Systematic and Evolution of *Peperomia* subgenus *Tildenia* (Piperaceae)

DISSERTATION

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Disputation am 07.07.2011

1. Gutachter: Prof. Dr. Christoph Neinhuis, Dresden
2. Gutachter: Prof. Dr. James F. Smith, Boise (USA)

[...] *es war der Trieb, eine wilde, großartige, an mannichfaltigen Naturprodukten reiche Natur zu sehen, die Aussicht, Erfahrungen zu sammeln, welche die Wissenschaften förderten.*

Alexander von Humboldt über seine Lateinamerikanische Expedition zwischen Peru und Mexiko (1799–1804)
(in: *Le voyage aux régions equinoxiales du Nouveau Continent, fait en 1799-1804*.).

[...] *I was anxious to contemplate a wild, stupendous nature rich on manifold produces and the hope of collecting experiences useful for the advancement of science.*

Alexander von Humboldt about his Latin American expedition between Peru and Mexico (1799–1804)
(in: *Le voyage aux régions equinoxiales du Nouveau Continent, fait en 1799-1804*.).

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INTRODUCTION

The genus *Peperomia* Ruiz & Pav.

Peperomia is a pantropically distributed genus, generally well known for its ephiphytic species contributing largely to the flora of the Neotropics. The few terrestrial species described so far, are notably outnumbered and often eclipsed by the manifold epiphytes of this plant group. However, recent studies indicate terrestrial lineages as the first successive branches in *Peperomia* (Wanke et. al, 2006; Samain, 2008; Samain *et al*., 2009).

The early molecular phylogenetic hypotheses have been the starting point of the present study. The position of the terrestrial clades is supported by an increased sampling in the course of this study (> 650 accessions) allowing a more representive overview of the life form pattern within the genus (Figure 1, based on ~350 accessions). At least six exclusively terrestrial lineages are succsessively arranged as sister to the remaining predominantly epiphytic clades of *Peperomia*. The current phylogentic pattern suggests that the terrestrial habit is ancestral. This finding in particular was interesting as major radiation within the genus is characterized by epiphytism (>80% of species are epiphytes). However characters leading to the epiphytic life form have probably evolved prior to the emergence of epiphytes in the evolution of *Peperomia*. Such characters might involve ecophysiological adaptions such as the possibility to tolerate unfavorable conditions. It is generally assumed that rainforest habitats are always humid but actually the substrate in rainforests canopies quickely dries even after a heavy rainfall and only species adapted to these quickly changing conditions are able to survive. Exactly these adaptations are found in at least two of the first terrestrial branches (Figure 1, clade A and F), which grow in very dry and/or seasonal habitats. Thus, these first evolutionary lineages can be regarded as most important in understanding the radiation of genus *Peperomia*. In addition, few terrestrial species appear scattered among the predominantly epiphytic clades and can be regarded as reversals. The accumulation of different life forms adapted to extreme habitats in a closely related plant group makes *Peperomia* an ideal group to study issues of character evolution and environmental adaptation.

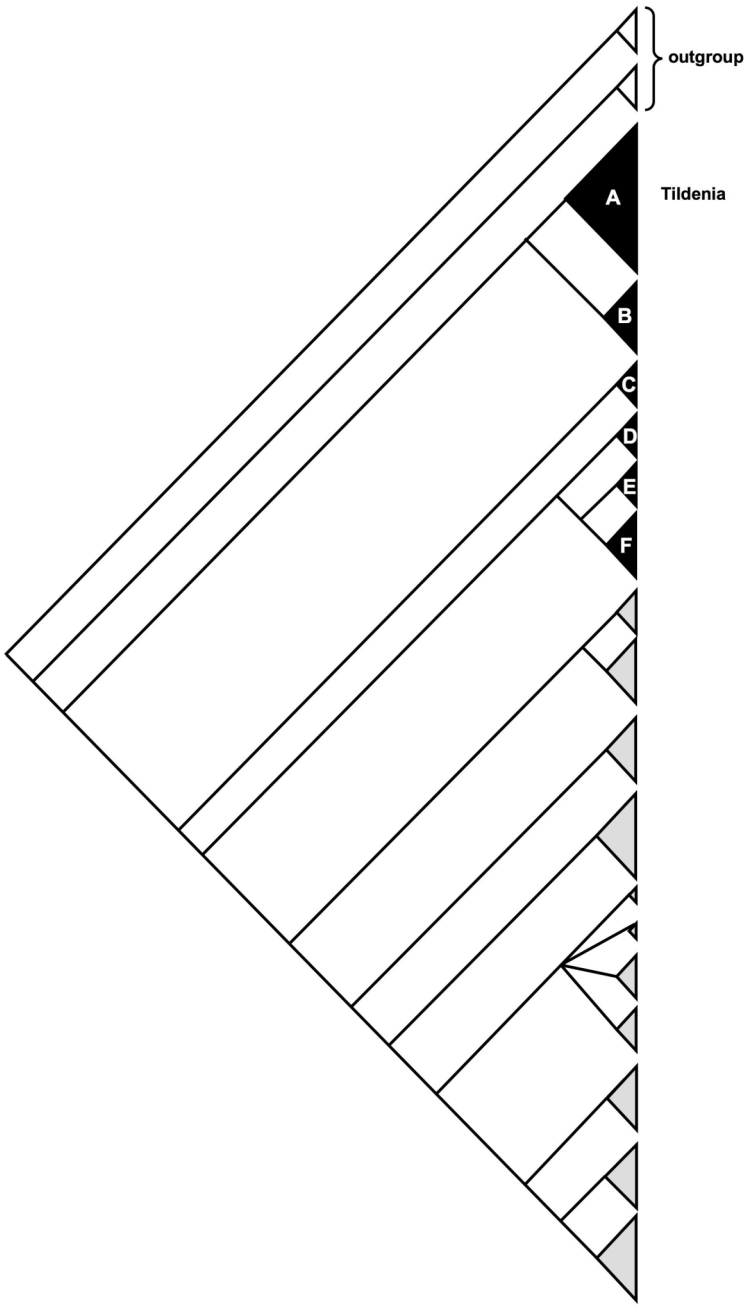


Figure 1. **Simplified phylogeny of *Peperomia* based on preliminary molecular analyses of 350 accessions. The topology derived from Bayesian inference of the chloroplast *trnK-psbA* gene region. Triangles symbolise a preliminary systematic concept of the genus based on groupings with significant statistical support. The size of the triangles corresponds to sampling size, not to total species numbers of the provisional subgenera. The black triangles indicate exclusively terrestrial groups, grey triangles are predominantly epiphytic.**

Peperomia contains approximately 1,650 species and belongs to the top ten of the most species-rich genera of angiosperms (Frodin 2004; Samain *et al*. 2009). By definition, a "giant genus" comprises more than 500 species (Frodin 2004), which today accounts for 57 angiosperm genera. According to the latest estimates, the total species number of all these genera makes up about 20 % of all vascular species worldwide (Sytsma & Walker 2005).

Although the proportion of "giant genera" on plant biodiversity is high, relatively few attempts have been made to study these groups comprehensively (e.g. Berry *et al*., 2005; Bruyns *et al*., 2006; Jaramillo *et al*., 2008). It is generally recognized that botanists should focus on resolution of these groups (e.g. Frodin 2004). However, the detailed knowledge required for treatment of the large amount of species is often impossible to achieve by only one scientist. To solve this problem, Sytsma & Walker (2005) proposed a "top down" approach in which large genera are subdivided in smaller monophyletic clades, which are then successively revised (Samain 2008).

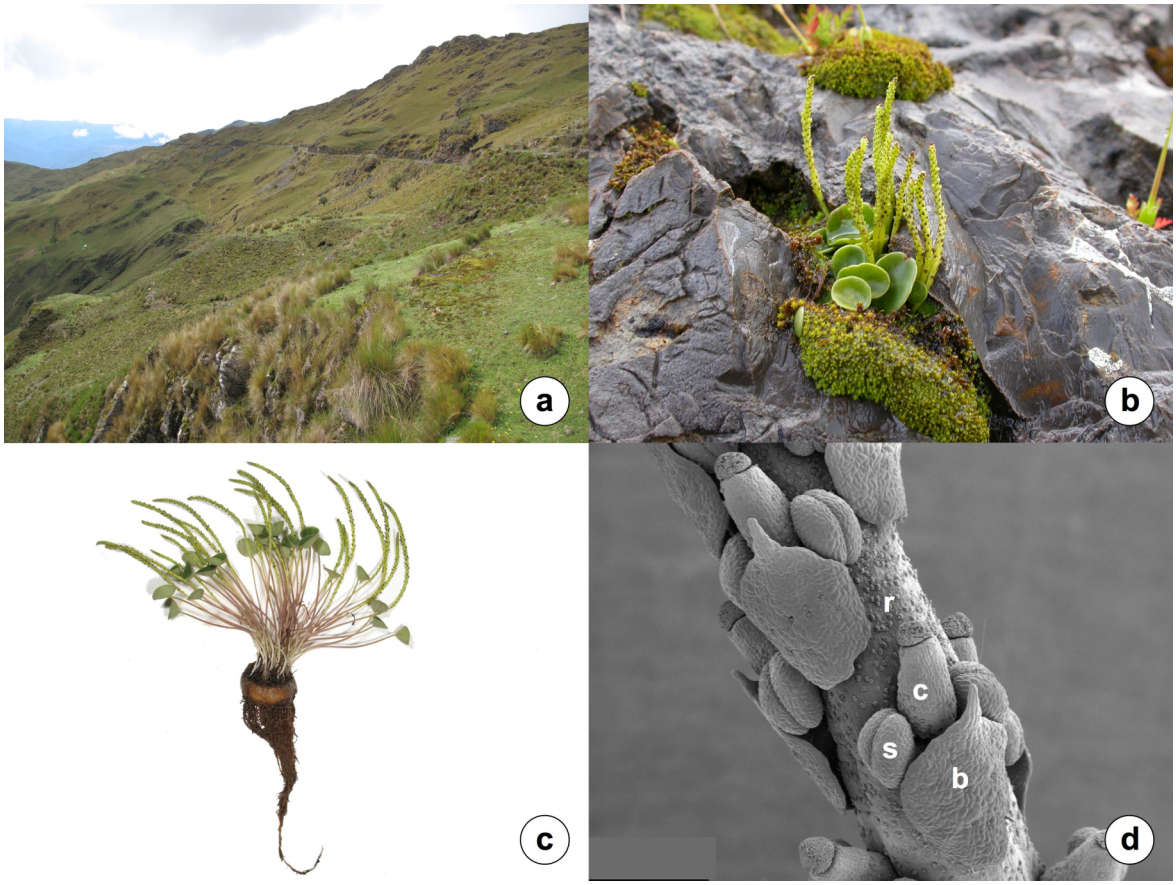
The only comprehensive systematic treatment of *Peperomia* including infrageneric relationships dates back more than 100 years (Dahlstedt, 1900). However, Dahlstedt's phylogenetic hypothesis did not correspond to the relationships mentioned in the descriptions of the subgenera/sections. The recent molecular studies (Wanke et. al, 2006; Samain, 2008; Samain *et al*., 2009) are the first attempts to resolve the chaotic situation within the genus. It could be shown that most of the characters used in *Peperomia* systematics so far are subject to parallel evolution. Thus, new synapomorphies matching the molecular hypotheses have to be found. Following the idea of the "top down" approach, the present study deals with one of the terrestrial lineages of the genus (Figure 1, A), the geophytic subgenus *Tildenia*, as starting point to reveal the evolutionary history of *Peperomia*.

***Peperomia* subgenus *Tildenia* (Miq.) Miq.**

The geophytic species of *Peperomia* underwent a taxonomical odyssey described in detail in chapter 2. Originally treated as discrete genus *Tildenia* (Miquel, 1843) the group was then transferred into *Peperomia* as subsection *Geophila* (Hill 1907) of subgenus *Tildenia* sensu Dahlstedt (1900). For more than a century this plant group has hardly been recognized. Only a few geophytic species of *Geophila* were newly described in the last decades (Trelease 1922, 1936; Rauh & Kimnach 1987; Pino 2004, 2008; Véliz 2007). Recent phylogenetic studies of *Peperomia* (Wanke et. al, 2006; Samain, 2008; Samain *et al*., 2009) and own unpublished data clearly show a separation of *Geophila* from the remaining species of *Tildenia* sensu Dahlstedt (1900). Since section *Geophila* contains the type species of subgenus *Tildenia*, *P. mexicana* (Miq.) Miq., this group will be treated as *Tildenia* hereafter. Together with another terrestrial clade (Figure 1, clade B), it represents the sister lineage to the remaining *Peperomia* species.

The distribution of *Tildenia* is mainly limited to two centres of diversity in Mexico/Guatemala and Peru/Bolivia. The species grow in (high) mountain areas (Figure 2a) with pronounced seasonal climate (Hill, 1906). Characters defining *Tildenia* from other species of *Peperomia,* are the formation of pronounced, mostly underground tubers with petioles and peduncles originating directly from it and peltate leaves (Figure 2c). The nature of the tuber (root-hypocotyl-stem or a combination) has not yet been investigated.

Although *Tildenia* is one of the most easily recognizable groups within *Peperomia* these species were largely unknown and undercollected in the past. The ephemeral life cycle is most likely the reason for the lack of knowledge of this plant group. Due to hibernation, the aboveground parts of these plants disappear completely for most of the year (~8 months). Leaves are only produced during the rainy season of the respective distribution area or when fog provides enough moisture. Moreover inflorescences and infructescences with mature fruits appear only for a very short span of the growing period. Furthermore, a comprehensive investigation of *Tildenia* was hindered by fragmented and insufficient documentation of the existing herbarium material.



Figur 2. **Characteristics of *Peperomia* subgenus *Tildenia*. a) Habitat of *P. polycephala*, Puna vegetation near Sorata (Bolivia). b) Yet unidentified species in its natural habitat. c) Habitus of *P. andina*. d) Highly reduced flowers typical for *Peperomia* on spadix of *P. cerrateae*: r=rachis, c=carpel, s=stamen, b=bract. Pictures by: a) Lars Symmank, b) and c) Marie-Stefanie Samain, d) Markus Günther.**

To obtain appropriate information for the treatment of *Tildenia* extensive fieldwork in both centres of diversity has been performed, complemented by important long-term observation by cooperating local botanists. This study is based on the analyses of ca. 300 own recent collections and approximately 1.000 additional specimens from 57 herbaria, which resulted in the first comprehensive analyses of *Tildenia* since more than 100 years.

Outline of this thesis

Chapter 1 focuses on the South American centre of *Tildenia* biodiversity. Initially a general rectified description of *Peperomia* subgenus *Tildenia* is presented based on the latest molecular analyses. Fourteen new species are described together with two formerly published varieties that are raised to species rank. Furthermore, for one name, basionym rejection and lectotypification are proposed. All valid species from the South American centre of diversity are treated in a phylogenetic framework.

A comparable study of the North American centre of diversity is presented in **Chapter 2** including a key to all *Tildenia* species from Mexico and Central America and the description of thirteen new species (one former variety). The new descriptions within this study doubles the species number of subgenus *Tildenia*. **Chapter 3** is a biogeographical study comprising the whole distribution area revealing the place of origin and the age of *Peperomia* subgenus *Tildenia.* The appearance of the major lineages of the subgenus is examined against the background of orogeny and climate changes in the respective distribution areas, to retrace the evolutional driving forces of this plant group. In **Chapter 4** *Tildenia* is used as a model group for the characterisation of a new nuclear single copy gene applicable for general use for phylogentic resoltution on species level and below. The search for suitable genetic markers to solve low level phylogentic relationships is essential for an elaborate treatment of *Tildenia*, *Peperomia* and multiple further plant and animal groups.

Contribution to the respective chapters

tasks	Chapter 2	Chapter 3	Chapter 4	Chapter 5
major paper writing			x	
part of paper writing	x	x		x
literature screening			x	
field work	x	x	x	x
molecular lab work	x	-	x	x
molecular data analysis & phylogenetics	x	-	x	x
Ancestral distribution and molecular dating reconstruction	-	-	x	-
distribution maps	-	x	x	-
illustration	x	x	x	

Not all tasks are applicable for the respective chapters (-)

CHAPTER 1

The South American centre of *Peperomia* subgenus *Tildenia* diversity

This chapter is a modified version of:

Samain, M-S., Mathieu, G., Pino, G., Symmank, L., Cieza, N., Neinhuis, C., Goetghebeur P. & Wanke S.

The geophytic *Peperomia* subgenus *Tildenia* (Piperaceae) in the Andes with the description of new species in a phylogenetic framework.

Plant Ecology and Evolution, 144, 148-176 (2011).

Summary

Background and aims

The pantropical genus *Peperomia* consists of approximately 1,600 species with an extremely diverse vegetative morphology, especially in the Neotropics. One of the most startling yet overlooked growth forms is the geophytic one, as in subgenus *Tildenia*. This group occurs in seasonal Neotropical habitats with its highest diversity in Mexico-Guatemala and Peru-Bolivia with few species known from in between these hot spots.

Methods

Recent fieldwork in Peru and Bolivia combined with herbarium study and a Bayesian analysis of an aligned sequence matrix of the chloroplast *trnK-matK-psbA* gene cluster of one accession of each species resulted in new findings within this subgenus.

Key results

Fourteen new species are described, discussed in a phylogenetic framework and illustrated. Two species have a wide distribution and occur from central W. Peru to S. Bolivia: the common *P. cerrateae* and the rare *P. parvisagittata*. Three endemic species are described from the Amotape-Huancabamba Zone which is particularly rich in tuberous *Peperomia* species: *P. gigantea*, *P. jalcaensis* and *P. klopfensteinii*. Two species are only known from their type locality in central W. Peru: *P. ocrosensis* and *P. pugnicaudex*. Five other Peruvian species are *P. ayacuchoana* from Ayacucho, *P. querocochana* from Ancash, *P. wernerrauhii* from Huánuco. *Peperomia umbrosa* from three distant localities, and *P. pseudoverruculosa* which is relatively common in SE Peru. Two species are Bolivian endemics: the rare *P. purpureonervosa* from La Paz and the more common *P. radiatinervosa* from Cochabamba and Chuquisaca. Two former varieties, both endemics of the extreme northern Central Andes, are raised to species rank: *P. lilliputiana* and *P. pseudoperuviana*. Finally, basionym rejection and lectotypification are proposed for the widespread and commonly collected tuberous *Peperomia* in the southern Central Andes: *P. peruviana*. An emended description of *Peperomia* subgenus *Tildenia* is also provided.

Introduction

With approximately 1,600 species, the pantropical genus *Peperomia* described by Ruiz and Pavón in 1794 from Peru belongs to the ten most species-rich genera of angiosperms (Frodin 2004, Samain et al. 2009). Among the 24 *Peperomia* species Ruiz & Pavón described in 1798 are two peculiar tuberous species, *P. umbilicata* and *P. scutellifolia*, both occurring in "loma" vegetation on very few localities along the Peruvian coast. They belong to what is now to be considered as *Peperomia* subgenus *Tildenia* (Miq.) Miq. ex Dahlst. (hereafter shortened as *Tildenia*), one of the most easily recognizable infrageneric *Peperomia* clades. This clade consists of approximately thirty currently accepted geophytic species with two easily observable synapomorphies: an entirely or partially underground tuber, and with petioles and peduncles originating directly from the tuber (Wanke et al. 2006, Samain et al. 2009). All these species occur in seasonal habitats of Neotropical mountain areas from Mexico to Argentina, with an almost equally high diversity in Mexico and Guatemala in the northern hemisphere and in Peru and Bolivia in the southern hemisphere. In between these two hotspots, there are only a few species known from a limited number of localities.

Tildenia was originally described by Miquel (1843a) as a separate genus -- then consisting of one species, *Tildenia mexicana* Miq. (accepted name = *Peperomia mexicana* (Miq.) Miq.) nom. cons. prop. (Mathieu 2009: 1374) -- within the family Piperaceae. Shortly afterwards, Miquel (1843b) transferred *Peperomia umbilicata* Ruiz & Pav. to the genus but named it *Tildenia peruviana* Miq. (see also below). In his Systema Piperacearum, he recognized *Tildenia* as a subgenus ['sectio'] of genus *Peperomia* within the tribe Peperomieae (Miquel, 1843c-1844). Dahlstedt (1900) recognized a much more widely circumscribed subgenus *Tildenia*, whereas Hill (1907) placed the tuberous geophytic species within the subsection *Geophila* of the subgenus *Tildenia* sensu Dahlstedt (Samain et al. 2007). However, recent phylogenetic analyses of the genus *Peperomia* based on coding and noncoding plastid DNA show that *Geophila* is clearly separated from the remaining species of *Tildenia* sensu Dahlstedt (1900), forming a distinct monophyletic clade which correctly should be named as *Peperomia* subgenus *Tildenia* (Miq.) Miq. (Wanke et al. 2006, 2007, Samain et al., 2007, 2009).

The last detailed studies dealing exclusively with *Tildenia* date back more than a century (Hill 1906, 1907). First, Hill (1906) published a detailed description of the morphology and seedling structure of this group. Next, he treated twelve South American and ten Mexican species (Hill 1907), some of which were later excluded or synonymized. Since this revision (Hill 1907), six taxa belonging to *Tildenia* in South America were newly described (Trelease 1936, Rauh & Kimnach 1987, Pino 2004, 2008, Pino et al. 2005). Similar to the

species described in this paper, all species (except *P. polycephala* Trel.) were based on fresh field collections, generally made by the author himself, and clearly are separate species. These species are not only supported by distinct morphological characters but also by our ongoing molecular investigations, which in addition confirm the relatively narrow species concept which is applied in the whole genus *Peperomia*.

The similar habit of many *Tildenia* species hinders their identification and makes observations of living plants in their natural environment, as well as meticulous reinvestigation of existing herbarium material, a prerequisite for correct identification. Indeed, since the phenotype within *Tildenia* is superficially very similar, many collections were often identified under a few common better known names, which led to a wrong estimation of species number and distribution. A comprehensive reinvestigation of this group has largely been prevented by fragmentary and insufficiently documented herbarium material. The study of *Tildenia* especially suffers from 1) fragmentary herbarium specimens with missing tubers or inflorescences, 2) the plants being above-ground for part of the year only, usually during the rainy season, and 3) the plants being quite small and inconspicuous. The latter two problems also account for the fact that this group has been overlooked and undercollected allover its distribution area. Field work and observation of the plants during their life cycle in a living collection is necessary for accurate description of the characters of each species.

In close collaboration with local botanists, field work, dedicated nearly exclusively to this group, was carried out in the past years in all countries of high diversity of *Tildenia*. In total more than one year of intensive field work in South America has been accumulated by the authors of this paper, complemented by important long-term observation by local collaborators. The information obtained from intensive fieldwork in combination with screening of available herbarium specimens allowed for a detailed treatment of the species of Bolivia and Peru and the discovery of species new to science. During this study it became obvious that subgenus *Tildenia* in the Andean hotspot includes much more species than initially assumed. In this study fourteen species are newly described and shortly discussed in a phylogenetic framework, two former varieties are raised to species rank and for one name, basionym rejection and lectotypification are proposed. An emended description of Peperomia subgenus Tildenia is also provided.

Materials and Methods

Field work and herbarium study

During field work in Bolivia (2008) and Peru (since the 1990s, and more intensively in 2009-2010), specimens of different developmental stages and longitudinal sections of tubers were preserved. When possible, infructescences with mature fruits were stored in 70 % alcohol. After careful cleaning and digital imaging of all aerial and subterranean parts, plants were immediately pressed and dried using a hot air drier that kept the temperature of the specimens below 50°C. This study is based on 300 of our own recent collections and approximately 400 additional specimens from 57 herbaria (see acknowledgements for an enumeration of these herbaria).

Sampling for molecular phylogeny

Our molecular phylogeny includes all currently known species of subgenus Tildenia reported from South America, with the exception of *P. minuta* Hill due to its uncertain taxonomic status. Whenever possible we used material from the type locality of the respective species. The outgroup consists of *P. pinoi* G.Mathieu, belonging to a yet unnamed subgenus closely related to Tildenia, *Piper crocatum* Ruiz & Pav. from the sister genus of Peperomia, and *Saururus chinensis* from Saururaceae, sister family to Piperaceae (Wanke et al. 2007). The plant material used for DNA extraction mainly comes from living plants collected in the field. Freshly harvested leafs where directly dried in silica gel. If silica dried material was not available, sampling was completed by material originating from herbarium specimens. The taxa used to generate the molecular data are listed in Table 1 with detailed origin information, and Genbank accession numbers.

Table 1. **List of sampled taxa included in the present study.**

Species	Lab number	Voucher	Field/garden origin (country, state/department)	Geographical coordinates	Altitude	Genbank accession
P. andina Pino	284	Pino 284 (USM)	Perú, Cajamarca	7°10'23"S 78°31'51"W	3050 m	HQ331483
P. ayacuchoana Pino & Samain sp. nov.	Pe428	Samain et al. 2009-040 (BR, GENT, USM)	Perú, Ayacucho	12°56'37,0"S 74°01'15,0"W	3190 m	HQ331493
P. cerrateae Pino sp. nov.	Pe408	Samain et al. 2009-002 (BR, GENT, USM)	Perú, Lima	11°26'51"S 76°36'57"W	2750 m	HQ331484
P. chutanka Pino	Pe436	Samain et al. 2009-076 (GENT, USM)	Perú, Lima	12°09'17"S 76°23'40"W	3320 m	HQ331485
P. cyclaminoides Hill	Pe110	Samain et al. 2008-080 (BR, GENT, LPB)	Bolivia, Tarija	21°09'16,5"S 64°41'32,0"W	2940 m	HQ331486
P. dolabella Rauh & Kimnach	885	Pino 885 (USM)	Perú, Cajamarca	7°12'19"S 78°30'24"W	3000 m	HQ331487
P. gigantea G. Mathieu & Pino sp. nov.	Pe358	Mathieu et al. 2009-158 (BR, GENT, USM)	Perú, Cajamarca	07°09'25,5"S 78°50'30,4"W	1770 m	HQ331488
P. jalcaensis Pino sp. nov.	Pe394	Mathieu et al. 2009-120 (GENT)	Peru, Cajamarca	07°11'19,8"S 78°34'32,3"W	3639 m	HQ331489
P. klopfensteinii G. Pino sp. nov.	Pe397	Mathieu et al. 2009-130 (BR, GENT, USM)	Perú, Cajamarca	06°39'32,3"S 78°44'01,4"W	2450 m	HQ331490
P. lilliputiana (Pino & Cieza) Pino stat. nov.	Pe361	Mathieu & Symmank 2009-024 (BR, GENT, USM)	Perú, La Libertad	07°53'03,1"S 78°07'21,2"W	3350 m	HQ331491
P. macrorhiza Kunth	RM275	Pino 327 (USM)	Perú, Cajamarca	7°11'24"S 78°21'27"W	2800 m	HQ331492
P. ocrosensis G. Mathieu sp. nov.	Pe355	Mathieu *et al.* 2009-042 (USM, BR, GENT, K, MO)	Perú, Ancash	10°27'32,4"S 77°24'29,6"W	3104 m	HQ331494
P. parvifolia C. DC.	Pe420	Samain et al. 2009-027 (GENT)	Peru, Huancavelica	12°20'40,8"S 74°56'13,0"W	4180 m	HQ331495
P. parvisagittata G. Mathieu & Pino sp. nov.	Pe352	Mathieu & Symmank 2009-020 (BR, GENT, USM)	Perú, Ancash	10°09'31,4"S 77°21'13,3"W	3480 m	HQ331496

P. peruviana Dahlst.	Pe168	Symmank et al. 2008-036 (BR, GENT, LPB, MO)	Bolivia, La Paz	15°44'14,8"S 68°41'54,0"W	2250 m	HQ331497
P. polycephala Trel.	Pe446	Samain et al. 2009-113 (BR, GENT, USM)	Perú, Cuzco	13°20'33,7"S 71°56'40,3"W	2950 m	HQ331498
P. pseudoperuviana (Pino) Pino stat. nov.	P151	Pino 894 (USM)	Perú, Cajamarca	7º17'31"S 78º29'27"W	2370 m	HQ331499
P. pseudoverruculosa G. Mathieu sp. nov.	Pe432	Samain et al. 2009-052 (BR, GENT, USM)	Perú, Ayacucho	13°18'55,7"S 73°58'48,8"W	3450 m	HQ331500
P. pugnicaudex Pino sp. nov.	Pe412	Samain et al. 2009-007 (BR, GENT, USM)	Perú, Lima	11°44"24,4"S 76°16'48,7"W	3430 m	HQ331501
P. purpureonervosa G. Mathieu sp. nov.	Pe174	Symmank et al. 2008-051 (BR, GENT, LPB, MO)	Bolivia, La Paz	16°18'54.8"S 67°55'28,4"W	3270 m	HQ331502
P. querocochana G. Mathieu & Pino sp. nov.	Pe372	Mathieu et al. 2009-043 (BR, GENT, MO, USM)	Perú, Ancash	10°20'08,1"S 77°20'88,0"W	3956 m	HQ331503
P. radiatinervosa G. Mathieu sp. nov.	Pe121	Samain et al. 2008-090 (BR, G, GENT, K, LPB, MO)	Bolivia, Chuquisaca	19°05'11,2"S 65°13'14,5"W	2900 m	HQ331504
P. rupiseda C. DC.	Pe413	Samain et al. 2009-008 (GENT, USM)	Perú, Lima	11°08'52,8"S 76°21'25,6"W	2680 m	HQ331505
P. scutellifolia Ruiz & Pav.	Pe579	Pino 2439 (USM)	Perú, Arequipa	15º46'56"S 74º23'13"W	1016 m	HQ331506
P. umbilicata Ruiz & Pav.	Pe324	Pino 1375 (USM)	Perú, Lima	11°21'17"S 77°21'56"W	590 m	HQ331507
P. umbrosa G. Mathieu sp. nov.	Pe395	Mathieu et al. 2009-122 (BR, GENT, USM)	Perú, Cajamarca	07°11'29,7"S 78°34'48,0"W	3557 m	HQ331508
P. verruculosa Dahlst. ex Hill	Pe456	Samain et al. 2009-123 (BR, GENT, USM)	Perú, Cuzco	13°30'08,3"S 71°58'48,2"W	3660 m	HQ331509
P. wernerrauhii Pino & Samain sp. nov.	Pe482	Samain et al. 2009-214 (BR, GENT, USM)	Perú, Huánuco	09°53'36,8"S 76°09'03,2"W	2470 m	HQ331510
Outgroup						
Piper crocatum Ruiz & Pav.		Wanke 070 (DR)	BG Bonn, 18143	-	-	DQ212714
Saururus chinensis Hort. ex Loudon		Wanke 001 (DR)	BG Bonn, 00312	-	-	DQ212713
P. pinoi G.Mathieu aff.	Pe474	Samain et al. 2009-230 (GENT, USM)	Peru, Huánuco	12°22'02,4"S 74°48'20,7"W	3560 m	HQ331482

DNA-isolation, amplification, and sequencing

We extracted genomic DNA following the modified miniprep procedure (Liang & Hilu 1996), published in Borsch et al. (2003). The final cleaning steps by ammonium acetate and sodium acetate were replaced by a single purification by columns using the NucleoSpin®-Extract II kit (MachereyNagel). We amplified the whole gene cluster (trnK intron, matK gene and the trnK-psbA spacer) in two parts with an overlapping region of 200 to 400 bp depending on the primer sets employed (Table 2).

The Polymerase Chain Reaction (PCR) consisted of an initial denaturation for 5 min at 94°C and 34 cycles of the following steps: 1) denaturation for 1 min at 95°C, 2) annealing for 1 min at 48°C, 3) elongation for 2 min at 68 °C. Finally, the reaction was treated at 68°C for 20 min and subsequently stored at 4 °C. The PCR was carried out in 50µl reactions, containing 1 µl DNA template of total genomic DNA (~ 10--50 ng), 10 µl dNTP mix (1.25mM each), 2 µl of each primer (20 pmol/µl) and 1 µl Taq Polymerase (PeqLab). After gel electrophoresis we cleaned the PCR products by NucleoSpin®-Extract II purification kit (MachereyNagel). The DNA sequencing was carried out on a Perkin Elmer automated sequencer (ABI Prism 377) or by Macrogen Inc. sequencing service (Macrogen, Seoul, Korea). We manually edited and aligned the sequences using PhyDE® (Müller et al. 2005).

Table 2. Primers used in present study.

Primer	Direction	Sequence 5´-3´	Reference
MG15	for.	ATCTGGGTTGCTAACTCAATG	Liang & Hilu (1996)
Pe-matK-2000F	for.	TTCCTTACGAATCCATAGA	Wanke et al. (2006)
Pe-matK-2500R	rev.	TTCGCAATAAATGCAAAGAGG	Wanke et al. (2006)
Pe-matK-2140F	for.	ACTTTATCTATTTATGGCAATG	this study
Pe-matK-2340R	rev.	TTTTCTTTTGACATTGAACCAA	this study
psbA-R	rev.	CGCGTCTCTCTAAAATTGCAGTCAT	Steele (1995)

Phylogenetic analyses

Phylogenetic analyses were conducted on the aligned sequence matrix after exclusion of five small regions of uncertain sequence homology assessment in the noncoding parts (table 3). Additionally, indels were coded at the end of the sequence matrix applying the simple coding algorithm of Simmons & Ochoterena (2000), implemented in the program SeqState (Müller 2005). We used jModeltest 0.1.1 (Posada 2008) to detect the optimal evolutionary models for our sequence data. Bayesian inference was applied using MrBayes v3.1 (Ronquist & Huelsenbeck 2003) with the 'model of best fit', GTR+G (-lnL=10.479,161) for the sequence partition. Ten runs with four chains each were executed simultaneously, initiated from random starting trees. Four million MCMC generations were run with a sampling frequency of 100. We assessed the burn-in using the program Tracer v1.5 (Rambaut & Drummond 2009). After exclusion of the first 10 % of the sampled trees we constructed a majority rule consensus tree with posterior probabilities ≥ 0,5. The resulting phylogenetic hypothesis was graphically displayed using TreeGraph 2 (Stöver & Müller 2010; Figure 1).

Table 3. Position of hotspots excluded from analyses in the *trnK-psbA* alignment.

Hotspot	Position	
H1	406 - 409	*trnK5´*-intron
H2	444 - 452	*trnK*5´-intron
H3	510 - 518	*trnK*5´-intron
H4	2448 - 2467	*trnK*3´-intron
H5	2589 - 2594	*trnK*3´-intron
H6	2661 - 2664	*trnK*3´-intron
H7	2848 - 2855	trnK-psbA spacer
H8	2933 - 3052	trnK-psbA spacer

Results and Discussion

Species concept and morphological variation

The species concept in *Peperomia* is quite narrow and intraspecific variation has not been investigated. This narrow concept of morphospecies hinders research within the genus, as it is very difficult to get familiar with such a large number of (sometimes highly similar) species within a relatively short period of time. The large species number is the direct reason for maintaining the morphological species concept, as a different and perhaps more convenient concept could only be introduced after a review of the whole genus or of at least a well-delineated group within the genus, such as a subgenus (Samain 2008). However, this morphological species concept is not problematic in *Tildenia* as, compared to some other subgenera, it consists of a relatively small number of species which are generally easy to distinguish and key out on the basis of conspicuous macromorphological characters. According to our observations, intraspecific variation is also quite limited in this group and is mainly restricted to a change in tuber shape (e.g. branching) with increasing age. Nevertheless, it remains difficult to include such variation into some species descriptions as a consequence of the very slow growth rate of the tuber.

The biology of *Tildenia* is also entirely unknown. Whereas extensive research was performed for some common epiphytic *Peperomia* species, e.g. *P. macrophylla*, (Youngsteadt et al. 2008), terrestrial species have not yet been seriously investigated with regard to pollination, fruit distribution, etc., perhaps as a consequence of the generally accepted idea that most *Peperomia* species are epiphytes. Data on hybridisation and cytology are also not available for *Tildenia*.

Phylogeny and classification

Our phylogenetic hypothesis lends support to the morphological species concept employed within the Andean *Tildenia* species. Figure 1 shows the phylogenetic relationships based on the chloroplast *trnK-psbA* gene cluster of these species, each represented by one accession, the type specimen, or in case of previously described species, a collection from the type locality. Most of the currently accepted species and all described species hereafter are supported by these molecular data.

Hill (1907) distinguished four rankless groups in his subsection *Geophila* Hill, mainly based on tuber morphology: *Parvifoliae*, *Umbilicatae*, *Campylotropae* and *Rhizomatosae* (Samain et al. 2007). However, as two species are each mentioned in two different groups, it is likely he did not want to give any taxonomical value to these groups. Our results show that these groups do not correspond to any of the monophyletic clades

recognized based on the *Tildenia* molecular phylogeny. Instead, there is a geographical pattern with eight Andean clades consisting of all included South American species. In addition to this parallelism between phylogenetic relationships and distribution, there also is a morphological evolution pattern with regard to the position of the roots on the tuber. The first branching Andean clades (A--D in Fig. 1) mainly consist of species with tubers with roots growing at the apex or allover their surface and rarely at the base whereas it is exactly the opposite in the last branching clades (E-H in Fig. 1).

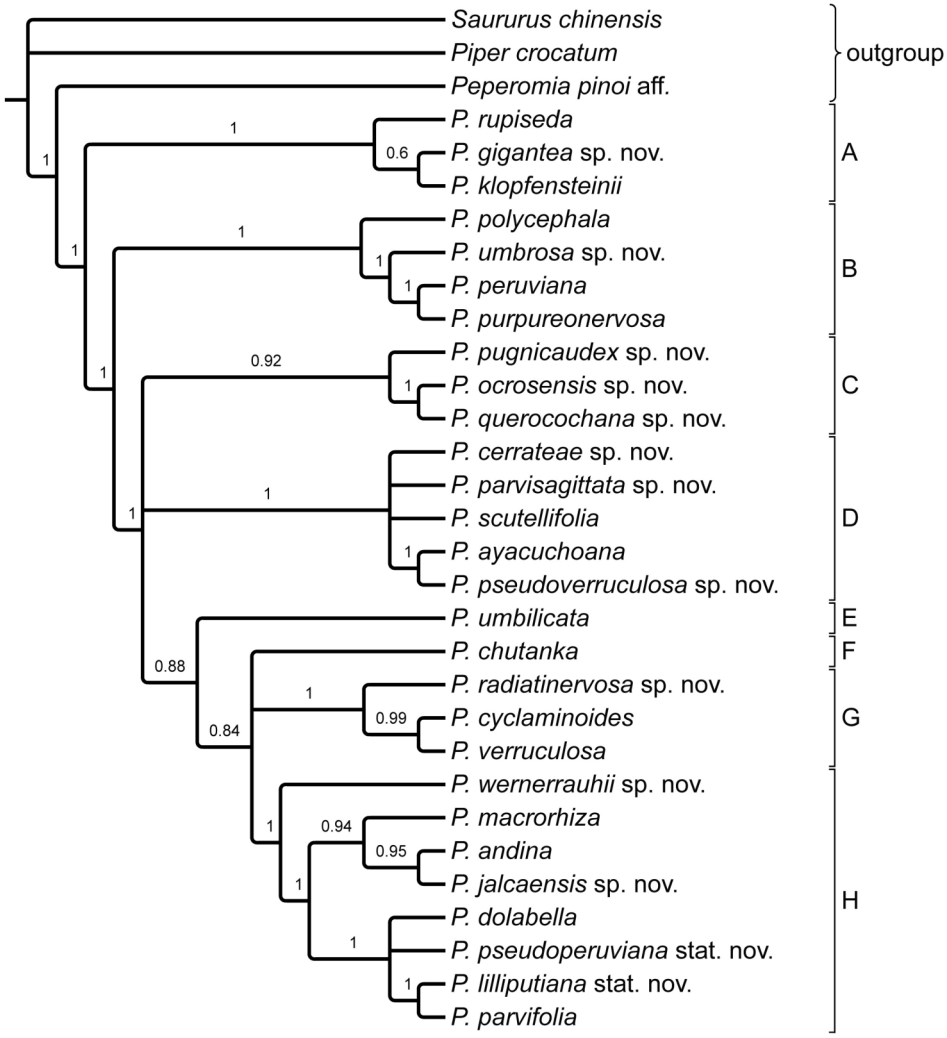


Figure 1. **Phylogenetic tree derived from Bayesian inference based on *trnK-psbA* gene cluster. The phylogentic position of the newly described species is shown with respect to the remaining South American species of *Peperomia* subgenus *Tildenia*. Posterior probabilities (PP) are shown above branches. See text for explanation of brackets along the tree.**

Distribution in the Andes

The diversity of *Tildenia* species is highest in the Central Andes, defined by e.g. Weigend (2002) as the area between the Huancabamba Depression in Northern Peru (at 4--6°S) and central Bolivia (near Santa Cruz, at 18 °S), followed by the Southern Andes (up to approximately 26°S in northern Argentina) and the northern Andes (between approximately 2°N in Ecuador up to nearly 11°N in Venezuela). All species included in the analysis of figure 1 occur in the Central Andes. The highest species diversity as well as the highest number of endemics of the clade is encountered in Cajamarca, S. Amazonas and La Libertad. This area coincides with the surroundings of the so-called Amotape-Huancabamba Zone, an east-west depression made by the Río Chamaya/Río Marañon drainage system. This zone forms at the one hand a biogeographic barrier to montane taxa between the Northern Andes and the Central Andes of Northern Peru, and at the other hand a biogeographic connection between the lowland forests of the Pacific coast and the Amazon basin through its lowest point at the Western Cordillera at the Paso de Porculla (2,145 meters). It is considered as an important biodiversity centre (e.g. Young & Reynel 1997, Knapp 2002, Stern et al. 2008, Weigend 2002, 2004, 2010a, 2010b).

Within the phylogenetic tree, a certain geographical pattern can be distinguished. The distribution core of clade A is in the souther portion of the Amotape-Huancabamba Zone with the rare species *P. klopfensteinii* and *P. gigantea* in the southern part and slightly south of this zone. The third species, *P. rupiseda* is wider spread and also ranges to the south until the department of Lima. Clade B consists of four species with main distribution ranging from central and southern Peru (*P. umbrosa* and *P. polycephala*) to Bolivia (*P. purpureonervosa* and *P. peruviana*) and up to northern Argentina (*P. peruviana*). Clade C consists of three rare species occurring in the western Cordillera in the Peruvian departments Lima and Ancash. Clade D does not show a clear geographical pattern. *Peperomia scutellifolia* is one of the two coastal loma species described by Ruiz and Pavón from the department of Arequipa. The other species in this clade are more widespread although *P. parvisagittata* is only known from three localities. Within this clade, *P. cerrateae* has a remarkably large distribution area ranging from central Peru to southern Bolivia. Branch E refers to the single species *P. umbilicata*, the second loma species from the Peruvian departments of Lima, Ancash and La Libertad. Branch F equally consists of the single species *P. chutanka* known from few localities of the department of Lima. Clade G consists of three species with core distribution of *P. radiatinervosa* and *P. cyclaminoides* from central to south Bolivia and *P. verruculosa* which is widespread in central and south Peru. Finally, clade H consists of eight species, six of which are restricted to the Amotape-Huancabamba Zone. The two other species do

not occur in this zone: *P. wernerrauhii* is known from the central Peruvian department of Huánuco whereas *P. parvifolia* occurs from central Peru to northern Argentina.

The species from the Amotape-Huancabamba Zone occur in different clades within the phylogeny, potentially indicating multiple parallel species radiations in this area. Also, the rest of the Central Andes is species-rich for *Tildenia* and has several rare endemic species scattered over the whole area. So far, we did not yet find a particular pattern in occurrence of these endemics. However, with the exception of the coastal species, *P. scutellifolia* and *P. umbilicata*, which grow on few remaining localities in the loma vegetation – habitats which are suffering heavily from human pressure – it should be emphasized that a large portion of the central Andes is undercollected for *Tildenia*. As a consequence, we do not yet know whether the pattern of narrow endemics we observe here is an artifact because of undercollecting or a genuine biogeographical pattern.

The Central Andes share several species with the southern Andes. Several of these are growing only in the southernmost portion of the Central Andes and the extreme northern part of the southern Andes, e.g. *P. cyclaminoides*, *P. radiatinervosa*. In contrast, some species have a much broader range, e.g. *P. parvifolia* and *P. peruviana*. The latter is probably the species with the southernmost occurence on the American continent, as it has been collected at up to 26° S in the Argentinian province Tucumán. *Peperomia peruviana* is sister to an endemic species from the Bolivian department La Paz, *P. purpureonervosa*. It is important to note here that the herbarium specimens of the northern Andes in Ecuador, Colombia and Venezuela which are identified as *P. peruviana* probably belong to a different yet undescribed species. However, recent attempts to collect this species in Colombia were not successful due to the extreme drought as a consequence of the El Niño weather phenomenon in the second half of 2009 and the first months of 2010.

Taxonomic treatment

The present treatment covers seventeen Andean species of *Peperomia* subgenus *Tildenia* of which fourteen new species, two former varieties which are raised to species rank and basionym rejection and lectoypification for one name.

Subgenus *Tildenia* (Miq.) Miq. ex Dahlst Dahlstedt 1900: 24. -- Type: *Tildenia mexicana* Miq., nom. cons. prop. (Mathieu 2009: 1374) (accepted name *Peperomia mexicana* (Miq.) Miq.)

Geophytic herbs with a perennial tuber and annual leaves and peduncles of inflorescences directly originating from the tuber.

Geophytic herbs, minute to large, 2--50 cm tall, entirely glabrous. Tuber entirely to partially buried, (sub)globose to ellipsoid, sometimes flattened or irregularly shaped, sometimes apically lobed, or rarely rhizomatous, rooting at apex, at base, or with roots all over its surface, whitish, yellowish or brownish. Leaves 1--20 per plant; petioles originating apically or rarely laterally on tuber; lamina often orbicular, occasionally (ob)ovate, cordate, deltoid, or reniform, membranous to coriaceous, rarely succulent, generally peltate, rarely non-peltate, often conspicuously palmatinerved. Inflorescences simple spadices, rarely compound; peduncles developing from the apical part of the tuber; spadices 1--20, peduncle and rachis generally slender, peduncle usually shorter than the often loosely flowered rachis. Floral bracts generally peltate, rarely non-peltate, orbicular, ovate or elliptic, apex rounded, acute or acuminate, occasionally glandularly dotted. Stamens with (sub)globose anthers, often very small, filaments short, occasionally long, caducous or persistent but wilting during fructification. Ovary ellipsoid, ovoid or conical, generally larger than the style. Style conical, ellipsoid, obovoid, mammiform or cylindrical, sometimes glandularly dotted. Stigma terminal, sometimes fimbriate, occasionally cone-shaped, sometimes on a stigmatal plateau, rarely flabelliform. Fruit (sub)globose, ellipsoid, ovoid, (sub)sessile or pedicellate, pericarp completely or partially granulose, (minutely) papillate or verruculose, rarely partially smooth.

The concept of *Peperomia* subg. *Tildenia* is here recircumscribed according to phylogenetic relationships determined by Wanke et al. (2006) and Samain et al. (2009). In contrast to Dahlstedt (1900), who expanded the circumscription to include all species with peltate leaves, the clade is restricted to the geophytic species exhibiting a perennial tuber from which petioles and peduncles of seasonal leaves and inflorescences directly originate. As such, the current circumscription largely corresponds to subsection *Geophila* A.W. Hill (1907). *Peperomia cotyledon* Benth. is excluded while the species described herein are included.

1. *Peperomia ayacuchoana* Pino & Samain, **sp. nov.**

Planta tubere globoso obovoideo supra excrescentia more capituli obtecto, foliis petiolatis peltatis rotundatis, spadicibus longissimis laxifloris pedunculo rachidi aequanti. Differt a *P. peruviana* Dahlst. forma, magnitudine coloreque tuberis, stylo quoque doliiformi (in illa elongato); a *P. pseudoverruculosa* G.Mathieu proxima, sed differt tubere majore rubrobrunneo, foliis majoribus et fructibus haud verruculosis. **--** Type: Peru, Ayacucho, prov. La Mar, dist. Tambo, along road Ayacucho--San Francisco, km 65--66, near Tambo, on rocks, 12º56'37"S, 74º01'15"W, 3,190 m, 24 Jan. 2009, *Samain et al.* 2009-040 (holo-: USM; iso-: BR, GENT).

Small herb, 9--16 (--20) cm tall. Tuber entirely buried, globose to obovoid, light reddish brown, obscurely tessellate, 0.6--1.5 x 0.5--1.7 cm diameter, base rounded, rooting at apex, which forms an irregular hemispherical excrescence, 0.4--0.9 x 0.6--1.3 cm diam, developing with age. Leaves 3--7 per plant; petiole 3--6 cm x 0.9--1.0 mm, terete, light green with reddish longitudinal lines near base, lighter and slightly thicker near insertion to lamina, erect to slightly curved; lamina orbicular, coriaceous, flat to slightly concave, centrally peltate, 1.2--1.9 cm diam, margin entire, adaxially glossy green, nerves obscure, abaxially greenish white, only main nerve conspicuous. Spadices 6--17 per plant, initially erect, then slightly curved; peduncle 3.5--9 cm x 0.8--0.9 mm diameter, light green or pink near base; rachis 4--9 cm x 0.9--1.1 mm diameter, light green, loosely flowered. Floral bracts elliptic, 0.7--0.8 x 0.5--0.6 mm, peltate, dark green, brown when old. Stamens with filaments 0.1--0.2 mm long, terete, white; anthers subglobose 0.3--0.4 mm wide, white when young, dark brown when drying. Ovary widely ovoid, 0.7 x 0.5 mm, bright green to brownish, stigma brown. Fruit ovoid, body 0.8--0.9 x 0.6--0.7 mm; pericarp papillate, shiny dark brown; style cylindrical to conical, 0.15--0.25 x 0.25--0.35 mm, brown. Fig. 2.

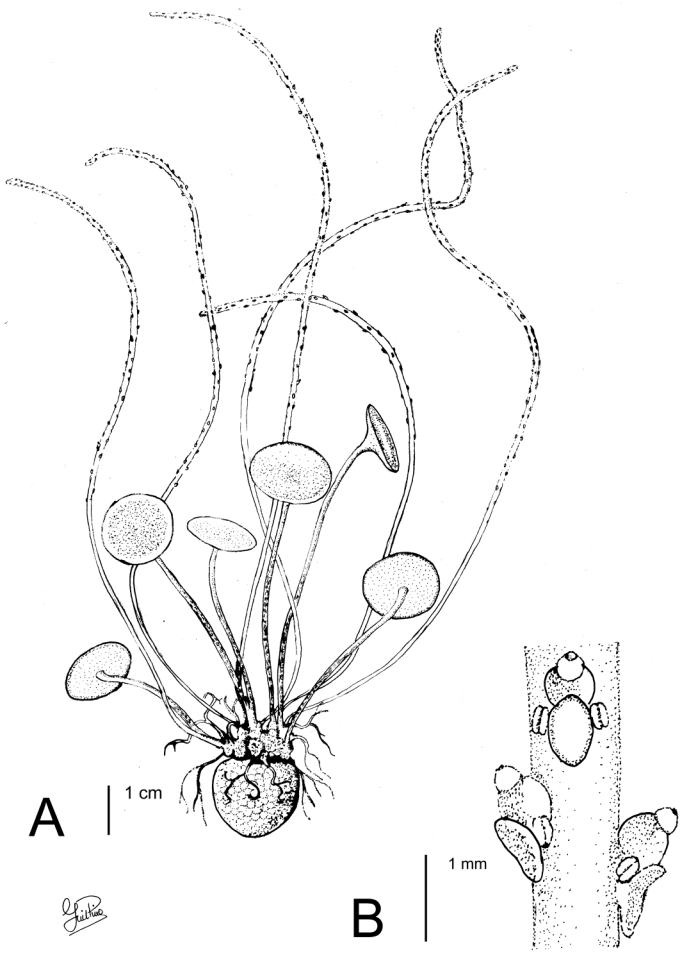


Figure 2. ***Peperomia ayacuchoana*: A, general habit; B, part of fruiting rachis.**

Other representative specimens examined -- Peru: Ayacucho, prov. La Mar, Dist. Tambo, along road Ayacucho--San Francisco, km 65--66, near Tambo, 12°56'37,9"S, 74°01'16,8"W, 3,180 m, 24 Jan. 2009, *Samain et al.* 2009-041 (GENT, USM); loc. cit., km 72, 12º55'51"S, 74º00'19"W, 3,290 m, 24 Jan. 2009, *Samain et al.* 2009-042 (GENT, USM); loc cit., km 64, 12º57'05"S, 74º01'21"W, 3,410 m, 24 Jan. 2009, *Samain et al.* 2009-044 (GENT, USM); prov. San Miguel, "Hatun Wayqo" 50 km E of Ayacucho, road to the jungle, 27 Mar. 2003, *Pino* 1114 (Photos).

Etymology -- This species is named after the Peruvian department of Ayacucho, where it was collected first. Note: This name is different from *P. ayacuchona* Trel., which is a herbarium name referring to *P. serpens* (Sw.) Loudon (Mathieu 2007: 44).

Relationships -- This new species has a very particular apical outgrowth upon the tuber, from where roots, petioles and spadices grow. Plants resemble *P. peruviana* Dahlst. but this latter species has smaller irregularly globose tubers developing the upper appendage only in old plants, and its fruits have a prolonged style compared to the short, barrel like style of *P. ayacuchoana*. *Peperomia ayacuchoana* also resembles *P. pseudoverruculosa* G.Mathieu but has larger, reddish brown and minutely tessellated tubers, longer spadices and non verruculose fruits. According to our phylogenetic analysis, *P. ayacuchoana* is sister to *P. pseudoverruculosa* (Fig. 1).

Phenology -- Leaves are present from November to April, spadices appear from January to March, and fruits ripe from February to March.

Distribution and habitat -- This species has only been collected in the department of Ayacucho in rocky places where it tolerates sun exposure, drought and human disturbance.

2. *Peperomia cerrateae* Pino & G.Mathieu, **sp. nov.**

Planta geophila a *P. scutellifolia* Ruiz et Pavón similis sed differt rachidibus spadicum decumbentibus longioribus angustioribusque; ab ea crescit in locis frigidis simulque humidis in montibus altis apud 3,000--4,000 m, foliis spadicibusque tantum a Novembro ad Aprilem praesentibus. -- Type: Peru, Lima, prov. Huarochirí, Dist. Mariatana, Huancalasila, under rocks, 12º14'S, 76º23'W, 3750 m, 4 Apr. 1968, *Cerrate et al.* 4436 (holo-: USM 210992).

Small herb, 3--7 cm tall. Tuber entirely buried, globose, very light yellow, greenish when exposed to daylight, brown when old, smooth, rooting from the apex, base rounded, 0.6--0.9 cm diam. Leaves 4--8 per plant; petiole (2--)3--6(--8) cm x 0.7--1.4 mm, terete, whitish, straight to slightly curved; lamina orbicular, chartaceous, flat to slightly concave, centrally peltate, 9--20 mm long, 11--22 mm wide, base rounded to subcordate, very rarely with overlapping lobes, apex widely emarginate (90% of the leaves) or rounded, margin entire, adaxially glossy green, minutely pitted, minutely centrally umbilicate, obscurely 5--palmatinerved, abaxially greenish white, only central nerve conspicuous from insertion of petiole to apex. Spadices 4--9 (--14) per plant; peduncle 2--5 cm x 0.6--0.8 mm, whitish, initially peduncle erect with nutant rachis, then the whole inflorescence erect or slightly curved; rachis (1--) 6--9(--12) cm x 0.7--1.1 mm, very light green, flowered every 0.5--1 mm. Floral bracts 0.6--0.7 x 0.4--0.5 mm peltate, ovate, green, brown when old. Stamens with filaments 0.3--0.4 x 0.12 mm, terete, white; anthers subglobose 0.3--0.4 mm

diam, white when young, dark brown when drying. Ovary narrowly ovoid to conical, bright green, 0.5--0.6 x 0.3 mm; stigma white, flabelliform. Fruit ovoid, 0.5--0.6 x 0.4--0.5 mm; pericarp papillate, olive green-brownish; style narrowly cylindrical to conical, 0.15--0.25 x 0.08--0.09 mm, brown. Fig. 3.

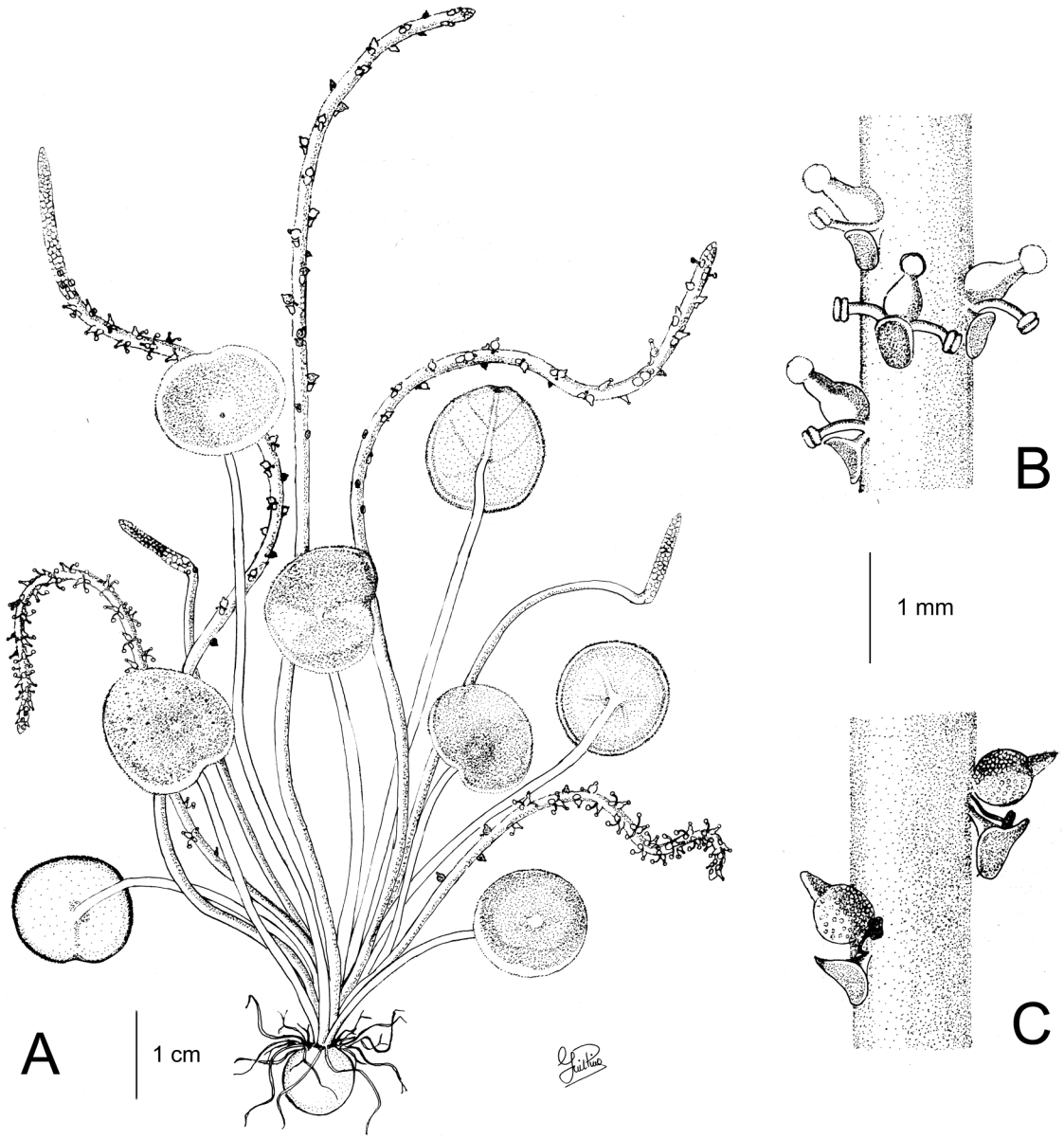


Figure 3. ***Peperomia cerrateae*: A, general habit; B, part of flowering rachis; C, part of fruiting rachis.**

Other representative specimens examined -- **Bolivia**: La Paz, prov. Inquisivi, along "Jucumarini Trail" between Chichipata and Jucumarini, Condor Conuña area, sandstone cliffs 2 km N of Quime, very fragile, growing entwined with the moss *Thuidum yungarum*, 16°58'S, 67°13'W, 3,300--3,400 m, 23 Feb. 1990, *Lewis* 37133 (LPB); prov. Larecaja, 11 km from Sorata on road to Consata, 15°43'40.9"S, 68°39'26.2"W, 3,200 m, 17 Jan. 2008, *Symmank et al.* 2008-041 (BR, GENT, K, LPB, MO); prov. Murillo, 1 km N (below) the dam of Lake Zongo, wet puna vegetation, at the base of rocks, 16°17'S, 68°07'W, 4,600 m, *Solomon* 15839 (LPB, MO); valley of the Río Zongo, 20 km N of the pass, shrubby vegetation (3--4 m) along creeks flanked by pastures, in moss mats in shady spots, 16°09'S, 68°07'W, 3,400 m, 17 Nov. 1987, *Solomon* 17433 (BR, LPB); Cochabamba, prov. Quillacollo, Cerro Tunari, 17°15'47.8"S, 66°20'59.3"W, 4,080 m, 12 Feb. 2008, *Samain & Bates* 2008-146 (GENT); Tarija, prov. Eustaquio Méndez, near León Canche, 21°06'50.2"S, 64°41'42.1"W, 3,000 m, 30 Jan. 2008, *Samain et al.* 2008-081 (BR, GENT). **Peru**: Ancash, prov. Bolognesi, dist. Cajacay, road Huaraz--Pativilca, km 103, 1 km above Majoralca, ca 250 m uphill Cerro Huansharury, 10º09'33"S, 77º21'06"W, 3,560 m, 25 Jan. 2009, *Mathieu & Symmank* 2009-021 (BR, GENT, USM); prov. Ocros, dist. Ocros, road Paramonga--Conococha (via Ocros), km 24, 10º20'31"S, 77º24'20"W, 2,360 m, 3 Feb. 2009, *Mathieu et al.* 2009-041 (BR, GENT, USM); road Huaraz--Paramonga, km 98, rock wall next to road, 10°00'32.7"S, 77°23'15.1"W, 2,975 m, 3 Feb. 2009, *Mathieu et al.* 2009-044 (BR, GENT, USM); Ayacucho, prov. Huamanga, dist. Acocro, along road Ayacucho--Chincheros km 79, 13º23'18.5"S, 73º57'38.1"W, 4,090 m, 26 Jan. 2009, *Samain et al.* 2009-055 (GENT, USM); along road Ayacucho--Chincheros km 82, 13º23'57.4"S, 73º56'52.3"W, 4,020 m, 26 Jan. 2009, *Samain et al.* 2009-056 (GENT, USM); loc. cit., *Samain et al.* 2009-057 (GENT, USM). Junín, prov. Chupaca, along road Huancayo--Yauyos, 27 km from Huancayo, 500 m before Roncia, 12º01'38"S, 75º24'29"W, 3,350 m, 21 Jan. 2009, *Samain et al.* 2009-016 (GENT, USM); Lima, prov. Huarochirí, dist. San Mateo, central highway km 98, beyond Infiernillo railroad bridge, N bank of Rimac river, before Cacray tunnel, aside waterfall, 11º44'21"S 76º16'45"W, 3,300 m, 29 Mar. 2002, *Pino* 836 (USM); central highway km 99, Anchi II bridge, W bank of Anchi river (flowing into the Rimac river from the S), on rocks, 11º42'46"S, 76º16'13"W, 3,680 m, 21 Mar. 2004, *Pino* 1294 (USM), 1446 (USM), 1650 (USM); E bank of Anchi river, 11º43'56"S, 76º16'05"W, 3,440 m, 18 Jan. 2009, *Samain et al.* 2009-006 (BR, GENT, USM); Dist. Chicla, central highway km 110, in small N exposed canyon, 11º40'26"S, 76º12'20"W, 3,900 m, 15 Dec. 2003, *Pino* 1254 (USM); central highway km 111, on rocks, 11º40'02"S, 76º14'51"W, 3,950 m, 1 May 2005, *Pino* 1441 (USM); central highway km 108, slope next to road and opposite church tower of Chicla, 11º42'16"S, 76º16'05"W, 3,790 m, 18 Jan. 2009, *Samain et al.* 2009-005 (BR, GENT, USM); Dist. San

Damián, road Santiago de Tuna--San Damián, before the town, on rocks, 12º00'22"S, 76º24'12"W, 3,200 m, 23 Jan. 2005, *Pino* 1581 (USM); Prov. Canta, Dist. Canta, above Obrajillo waterfall, 11º26'51"S, 76º36'57"W, 2,750 m, 17 Jan. 2009, *Samain et al.* 2009-002 (BR, GENT, USM); Dist. Huaros**,** road Canta--La Viuda pass, N detour towards Huaros, with *P. galioides,* 11º24'31"S, 76º33'05"W, 3,300 m, 19 Feb. 2005, *Pino* 1596 (USM); Dist. Lachaki, road Canta--Lachaki, banks of Chacallayqui river*,* 11º31'58"S, 76º37'11"W, 3,630 m, 12 Apr. 2008, *Pino* 1960 (USM); Prov. Oyón, Dist. Caujul, road Sayán--Caujul*,* 10º48'37"S 76º59'03.7"W, 3,170 m, 21 Mar. 2008, *Pino* 1936 (USM); Prov. Yauyos, Dist. Tomas, along road Huancayo--Yauyos, 103 km from Huancayo, 2 km beyond Tinco, 12°16'55.4"S, 75°42'06.4"W, 4,010 m, 21 Jan. 2009, *Samain et al.* 2009-023 (GENT, USM).

Etymology -- The specific epithet is chosen in honor of Emma Cerrate, collector of the type. She specialized in ethnobotany and is, after her husband Ramón Ferreyra, the Peruvian researcher who made the highest number of *Peperomia* collections in the 20th century, mainly in Peru.

Relationships -- At first sight, *P. cerrateae* resembles *P. umbilicata* Ruiz & Pav. However, *P. cerrateae* has larger leaves and inflorescences. It also has smaller fruits and white anthers instead of red or brown ones. The phylogeny shows this species to be in a polytomy of *P. scutellifolia*, *P. parvisagittata*, *P. ayacuchoana* and *P. pseudoverruculosa* (Fig. 1). The spadices of *P. scutellifolia* tend to be erect and are shorter than the long trailing spadices of *P. cerrateae*. *P. umbilicata* as well as *P. scutellifolia* differ in phenology, appearing above-ground during the South American winter months (May--August). These two species occur in dry W facing coastal mountains at relatively low elevations between 300--1,000 m.

Phenology -- Leaves are present from November to April, spadices appear from January to March, and fruits ripen from February to April.

Distribution and habitat -- This species is a widespread Andean geophyte occurring at elevations between 2,750--4,600 m, reported from the Peruvian department of Ancash down to the department of Tarija in Bolivia. It grows in clayish soil between rocks, together with mosses, in moderately shaded places.

3. *Peperomia gigantea* G.Mathieu, **sp. nov.**

A *P. rupiseda* C.DC. differt statura magna, bracteibus floralibus junioribus apice caudato, stylo lobato laevique, stigmate plano. -- Type: Peru, Cajamarca, Prov. San Pablo, Dist. San Bernardino, road Chilete – San Pablo, 23.9 km from Chilete, "Boladera La Cueva", rock wall 50 m left from the cave, 07°09'25.2"S, 78°50'30.4"W, 1,780 m, 28 Feb. 2009, *Mathieu & Symmank* 2009-158 (holo-:USM; iso-: BR, GENT).

Large herb, up to 50 cm tall when flowering. Tuber more or less globose, slightly flattened, usually small for size of plant, up to 2 cm diameter, surface yellow or greenish yellow when partially exposed to daylight, white on cross section, rooting at apex. Leaves 1--3 per plant; petiole (8--)15--20 cm, terete, whitish green to green or pinkish/purplish red, minutely longitudinally striate; lamina irregularly orbicular, (4--)6--10(--13) cm diameter, slightly undulate and minutely sunken in the middle, vivid green adaxially, dull whitish green abaxially, 8-palmatinerved, main nerves contrasting yellowish adaxially, not reaching margin. Spadices 1--5 per plant; peduncle up to 35 cm, terete, usually more reddish than petiole; rachis up to 15 cm, usually less than half length of peduncle, not wider than peduncle. Floral bracts elliptic, 1.5 mm long, apex caudate and comprising 1/2 to 2/3 of total length of young floral bract, long acuminate when growing older. Stamens with short, apically widening filaments; anthers ca 0.4 mm long. Ovary lighter than style, apical part of ovary covered by irregularly lobed, smooth cap; stigma terminal, usually flat, exceptionally divided. Fruit globose when young, mature fruit not seen. Fig. 4.

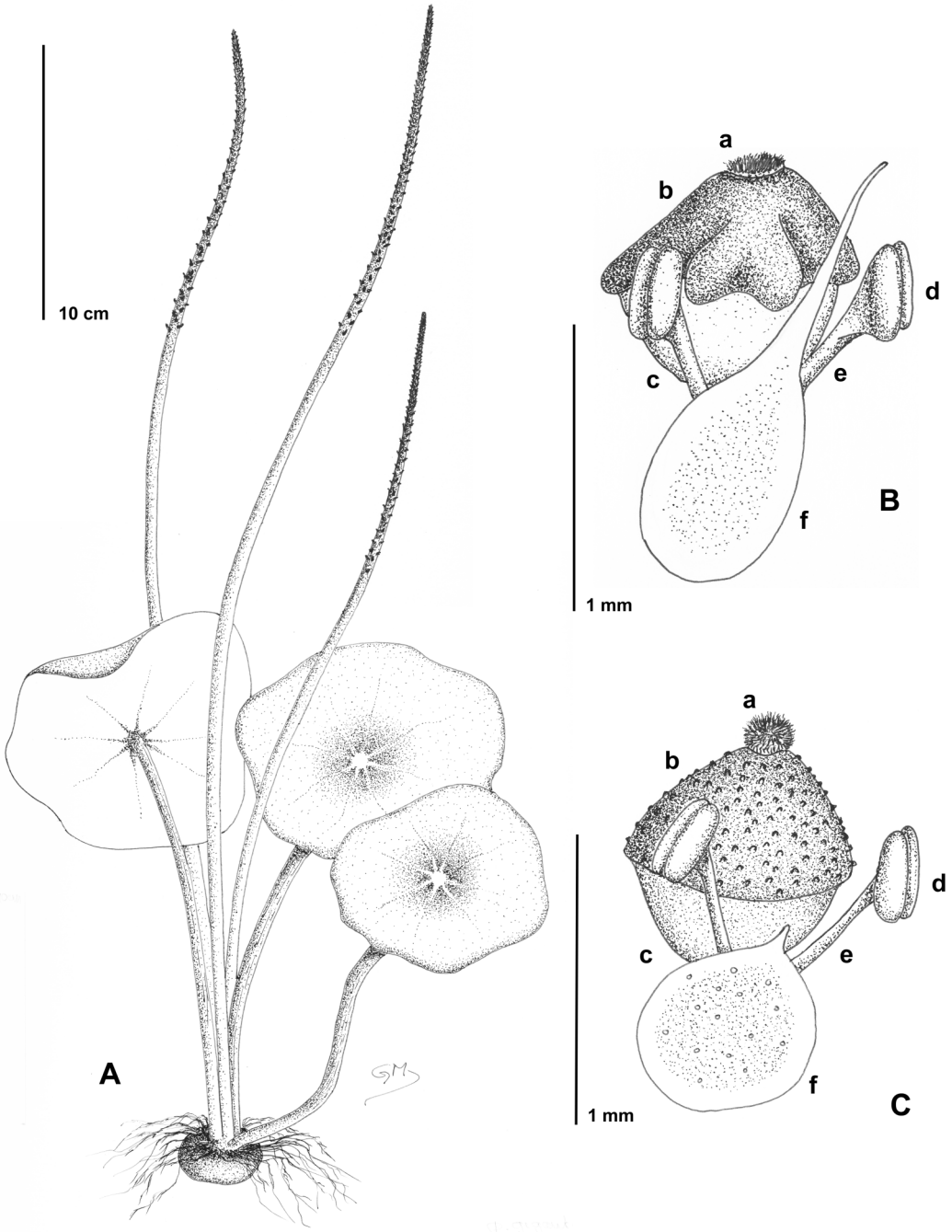


Figure 4. **A, B, *Peperomia gigantea*; C, *P. rupiseda*: A, general habit; B, flower; C, flower, a. stigma, b. style, c. ovary, d. anther, e. filament, f. floral bract.**

Other representative specimens examined -- **Peru**: Cajamarca, prov. Contumazá, road Cascas--Contumazá, Plantanar Electrical power plant, 1,400 m, 31 Mar. 1994, *Sagástegui et al.* 15197 (F, MO).

Etymology -- The specific epithet refers to the size of the species as it is the largest known geophytic *Peperomia*.

Relationships -- Small specimens of *P. gigantea* resemble *P. rupiseda* C.DC. Tubers of *P. gigantea* are solitary, yellow to greenish yellow and root from the apex whereas in *P. rupiseda* they tend to form clusters, are light brown and root from the base or occasionally from all over its surface. Both species show subglobose fruits, which differ however in the shape of their style. The differences are most clear in the young pistil before the style deforms and shrinks concurrent with the growth of the fruit. In *P. gigantea* it is an irregularly lobed, smooth cap usually bearing a flat stigma whereas in *P. rupiseda* it is a minutely papillate dome bearing a globose stigma. *P. gigantea* also differs in the young floral bract with distinct caudate apex. Based on molecular data, *P. gigantea* and *P. rupiseda* form a clade together with *P. klopfensteinii* Pino & Cieza (Fig. 1). Within this clade, *P. gigantea* appears to be related more closely to *P. klopfensteinii* than to *P. rupiseda*. The two known collections of *P; gigantea* are from 1,400 and 1,780 m, substantially lower than *P. rupiseda* which is commonly found above 2,500 m.

Phenology -- The plant has been found above-ground in February and March, flowering and with immature fruits.

Distribution and habitat -- *Peperomia gigantea* seems to be a rather rare species occurring in western Cajamarca whereas the more common *P. rupiseda* is reported from Ancash, La Libertad and Lima.

4. *Peperomia jalcaensis* Pino, **sp. nov.**

Planta geophila a *P. parvifolia* C.DC. differt spadicibus laxioribus, bracteis albidis antherisque rubris, fructibus angustioribus stylis pro ratione majoribus. A *P. macrorhiza* Kunth differt tubere infosso minore nec multilobato nec nitido, spadicibus brevioribus et fructibus longioribus. -- Type: Peru, Cajamarca, Prov. Cajamarca, Dist. Cajamarca, Cumbemayo, on rocky wall among mosses and foliose lichens, 07º11'19"S, 78º33'32"W, 3,650 m, 17 May 2001, *Pino* 663 (holo-:USM 184604).

Small herb, 3.5--4.5 cm tall above surface. Tuber usually buried among mosses in shadowed places, globose to depressed, grayish brown or yellowish, 1.2--2.3 cm high, 1.2--1.5 (--2.5) cm diam, apex flat with one to several buds, rooting at base. Leaves 7--9 per plant; petiole (0.8--)2.2--3.2 cm x 0.5-0.6 mm, terete, light green with reddish stripes or totally reddish, straight to slightly curved; lamina orbicular to subdeltoid, succulent, funnel-shaped, peltate a little below middle, 0.4--0.6 cm long, 0.35--0.5 cm wide, margin entire, obtuse to subemarginate, base rounded, adaxially concave, dark green, nerves not visible, abaxially greenish white, obscurely 5--7-palmatinerved. Spadices 8--20 per plant;

2--4 from each bud, erect to slightly curved; peduncle 2.8--3.5 cm x 0.6--0.9 mm, light green or with reddish stripes; rachis (1.4--) 1.8--2.4 cm long x 1.2--1.4 mm, light green or reddish when exposed. Floral bracts peltate, orbicular, acuminate, white, 0.6--0.7 mm diameter. Stamens with filaments 0.2--0.3 mm long; anthers subglobose 0.2--0.25 mm diam, dark red when young, then brown. Ovary ovoid, light green to reddish, 0.5--0.6 mm x 0.3--0.4 mm. Fruit narrowly ovoid, body 0.9--1 mm x 0.6--0.7 mm, brown, papillate; style widely conical with flat top, 0.2--0.3 mm long x 0.3--0.4 mm diam, dull yellowish green. Fig. 5.

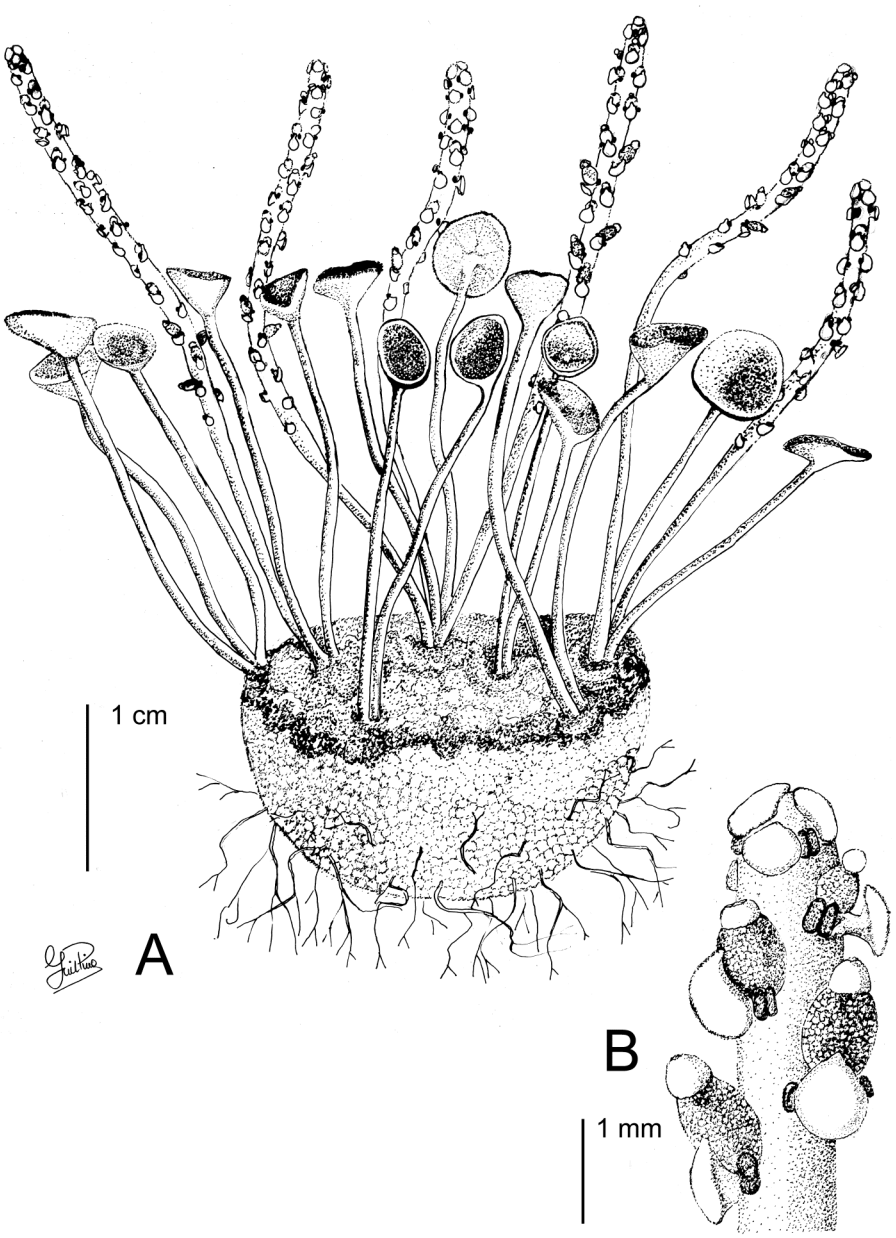


Figure 5. ***Peperomia jalcaensis*: A, general habit; B, part of flowering rachis.**

Other representative specimens examined -- **Peru**: Cajamarca, prov. Cajamarca, dist. Cajamarca, around the city of Cajamarca, 3,700 m, 31 May 1986, *Becker & Terrones* 1836, (USM 76415); Cumbemayo, ca 20 km SW of Cajamarca, on solitary rock, 07°11'19.8"S, 78°34'32.3"W, 3,640 m, 18 Feb. 2009, *Mathieu et al.* 2009-120 (BR, GENT, K, MO, USM); Cumbemayo, on rocky formations "Frailones", 07º11'17"S, 78º34'32"W, 3,500 m, 12 Apr. 2002, *Pino* 926 (USM); Maqui-Maqui mountain, N of Cajamarca, route to Shanta Alta, on grassy "Jalca" upon rocks, 3,900 m, 15 Jan. 1994, *Sánchez-Vega & Cabanillas* 6666 (CPUN 4314); lower slope of Baúl mountain, 3,900 m, 5 Feb. 1994, *Sánchez-Vega & Cabanillas* 6719 (CPUN 19137); prov. San Marcos, dist. Gregorio Pita, Las Pajas, 07º11'46"S, 78º09'60"W, 3,765 m, 10 Dec. 2002, *RRP* 360 (Photos).

Etymology -- The epithet stands for the habitat of this species: "jalca" is the common name of the moist rocky and shrubby highlands above 3600 m in northern Peru, which is intermediate between "páramo" from the northern hemisphere and "puna", the open, seasonally dry grassland above 4000 m from Central and Southern Peru, Bolivia and Argentina.

Relationships -- Several collections mentioned here were erroneously cited by Pino (2004: 31) as *P. parvifolia*. However, leaves in *P. jalcaensis* are somewhat more succulent, glossy, darker and funnel-shaped, with the petiole less excentrically inserted; spadices of *P. jalcaensis* are longer, less compact on anthesis, with white bracts and red anthers instead of green as seen in *P. parvifolia*. Fruits are somewhat smaller but much narrower in *P. jalcaensis* with a relatively larger style, light green in contrast.

Large tubers of *P. jalcaensis* could also be confused with *P. macrorhiza*, a species that grows in the same area although 1,000 m lower and in drier places. However, the tubers of *P. jalcaensis* are smaller, generally covered by organic matter, brownish, coarse, globose or discoid instead of the large, exposed, glossy grayish and flat irregularly lobed tubers of *P. macrorhiza* and *P. lilliputiana* (Pino & Cieza) Pino. Leaves in *P. macrorhiza* are usually orbicular and peltate at the middle, flat to slightly concave and very rarely funnel-shaped, in *P. jalcaensis* they are more succulent, consistently funnel-shaped and peltate below the middle. Spadices of *P. jalcaensis* are stouter, shorter and appear and remain at the same time as the leaves, similar to those of *P. lilliputiana*, while in *P. macrorhiza* they are longer, more laxly flowered and precede the leaves being short-lived. The most important difference is the shape of the fruit, which in *P. jalcaensis* is very

narrowly ovoid, with a large conical style, overall much longer than the fruits of the other species growing nearby that have mainly globose and smaller fruits (*P. macrorhiza* Kunth, *P. andina* Pino*, P. dolabella* Rauh & Kimnach*, P. lilliputiana* (Pino & Cieza) Pino). In the molecular phylogeny, *P. jalcaensis* is most closely related to *P. andina* (Fig. 1).

Phenology -- Leaves are present from November to May, spadices appear from December to May, fruits ripen from December to June.

Distribution and habitat -- This species occurs in "jalca" formations of the Peruvian department of Cajamarca where it grows on rocks with a very thin layer of substrate, together with mosses and lichens that hide the tubers.

5. *Peperomia klopfensteinii* Pino & Cieza, **sp. nov.**

Planta geophila tubere globoso apice radicibus instructo; folia longe petiolata lamina tenui iridiscenti, rotundata, apice acuto vel acuminato, sub medio peltata; spadices erectae staminibus longissimis antheris rubris; habitat in cryptis perumbrosis humidis Peruviae septentrionalis. -- Type: Peru, Cajamarca, prov. Hualgayoc, dist. Chugur, Perlamayo, road Hualgayoc--Chugur, very shaded, wet places on rocks, growing with *Masdevallia* sp., *Lycaste* sp., 06º39'16"S, 78º43'71"W, 2,557 m, 16 Nov. 2002, *Klopfenstein* RRP-343 (holo-: USM 230318).

Medium sized herb, 10--20 cm tall. Tuber entirely buried, globose to slightly flattened, yellowish brown to light brown, 1.2--1.6 cm diameter, rooting at apex. Leaves 2--5 per plant; petiole 5--8(--15) cm x 1.8--2 mm, terete, light green to pinkish flushed with red, straight to slightly curved; lamina orbicular to widely obovate, membranous, flat, peltate a little below middle, 4--8(--11) x 3--7 cm, apex obtuse to acute, frequently slightly acuminate, base rounded, margin entire, adaxially vivid green to iridescent, (5--)7--9-palmatinerved, nerves lighter, reticulately nerved in between, abaxially greenish white to reddish when more exposed, nerves same as adaxially but slightly darker. Spadices (1--)6--11 per plant; erect or slightly curved but tips erect; peduncle 3--5 cm x 1--1.4 mm, whitish or pinkish flushed with red; rachis 3.5--6 cm x 1.2--1.6 mm, light green to reddish, densely flowered. Floral bracts peltate, orbicular, 0.6 mm diameter, same colour as rachis, inconspicuous. Stamens with filaments 0.9--1.2 x 0.15--0.2 mm, terete, transparent; anthers subglobose 0.4--0.5 mm diam, reddish when young, then bright white. Ovary cylindrical, 0.7--0.8 mm x 0.3--0.4 mm, bright green; stigma white. Fruit not seen. Fig. 6.

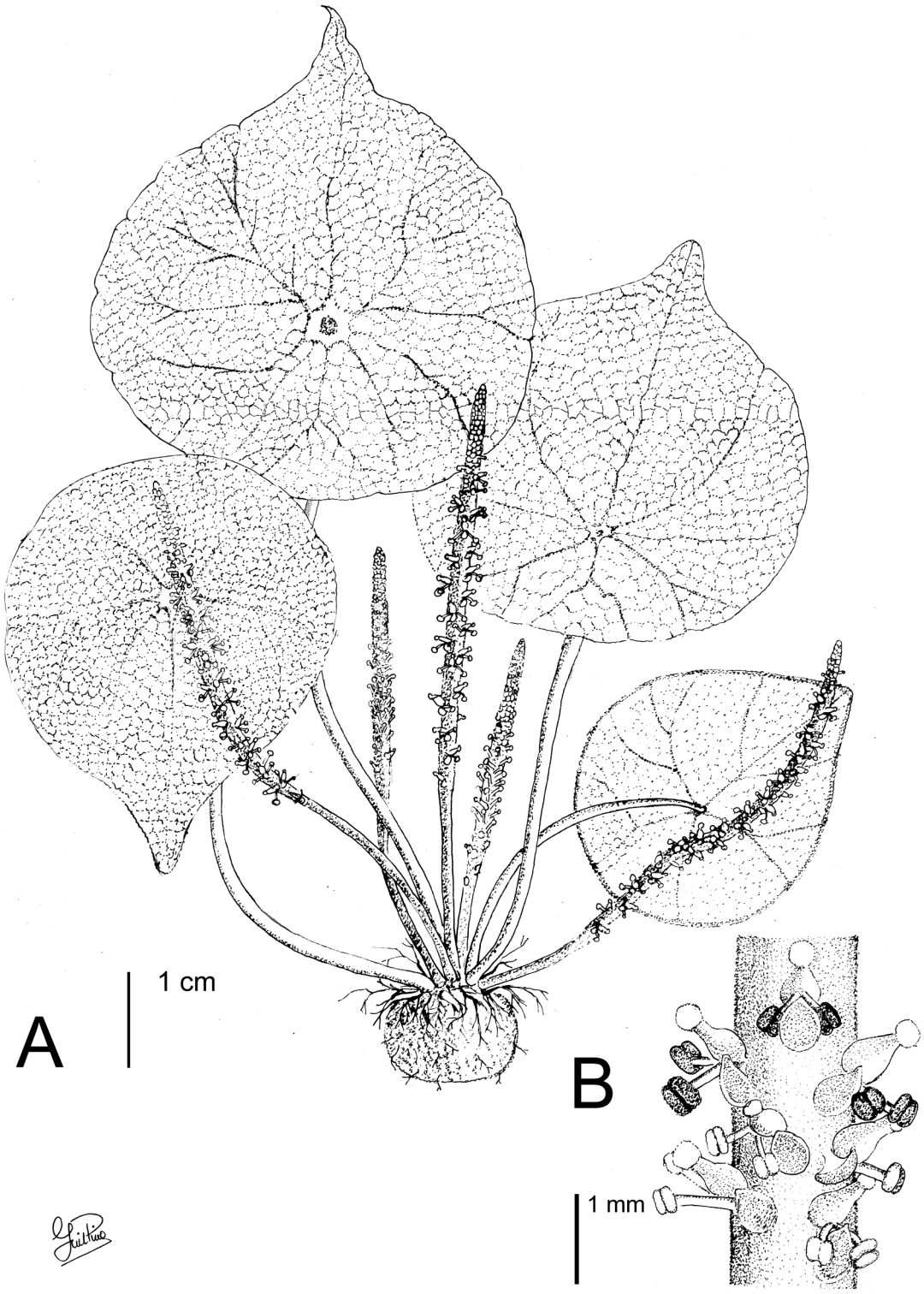


Figure 6. ***Peperomia klopfensteinii*: A, general habit; B, part of flowering rachis.**

Other representative specimens examined -- **Peru**: Amazonas, prov. Luya, dist. Lamud, 'Caverna de Quiocta', entrance to the cave, 06°08'21.0"S, 77°59'26.1"W, 2,800 m, 14 Feb. 2009, *Mathieu et al.* 2009-104 (BR, GENT, MO, USM); Cajamarca: prov. Chota, dist. Conchán, road Chota--Conchán, km 13,6, in cave along the road, 6°29'36,4"S, 78°37'26,9"W, 2,852 m, 4 November 2010, *Samain et al.* 2010-168 (GENT, USM); prov. Cutervo, dist. San Andrés de Cutervo, 2.5 km from San Andrés, "Gruta de Guacharo", at the entrance of the cave, dripping rock wall next to torrent, 06°13'44.4"S, 78°45'15.0"W, 1,550 m, 22 Feb. 2009, *Mathieu et al.* 2009-135 (BR, GENT, USM); prov. Hualgayoc, dist. Chugur, road Chugur--Hualgayoc, track to Rio Grande, 2.5 km from Chugur, 200 m downhill by trail, 30 m upstream from bridge, in side canyon, "La Palma", soil pockets on shady rock wall, 06°39'32.3"S, 78°44'01.4"W, 2,450 m, 20 Feb. 2009, *Mathieu et al.* 2009-130 (BR, GENT, MO, USM); prov. San Ignacio, Dist. Tabaconas, road Tabaconas--Huancabamba 40.9 km, at the entrance of very steep canyon with waterfall, in mosses on vertical rock wall, 05º19'28"S, 79º21'38"W, 2,670 m, 6 Feb. 2009, *Mathieu et al.* 2009-049 (BR, GENT, USM).

Etymology -- The species is named after Olivier Klopfenstein, collector of the type. He is a Swiss ranger and amateur botanist, founder of the Botanical Garden of San Marcos in Cajamarca, a tireless researcher of the Peruvian flora during his stay in Peru (1998--2004).

Relationships -- This species is delicate and easily disintegrates when touched. Leaves are large, papery, ovate, acuminate, easily breakable and with an iridescent colour. Spadices are conspicuous, straight or slightly curved but with erect tips, reddish rachides with very long stamens and red anthers. Based on molecular data, *P. klopfensteinii* is sister to *P. gigantea* G. Mathieu (Fig. 1).

Phenology -- Leaves present from November to April. Spadices appearing at same time as leaves.

Distribution and habitat -- The species thrives in very shady, humid places preferably near water courses, frequently at cave entrances or very steep canyons. It grows with mosses in clayish soil in rock crevices.

6. *Peperomia lilliputiana* (Pino & Cieza) Pino, **stat. nov.**

Peperomia macrorhiza var. *lilliputiana* Pino & Cieza, Haseltonia 11: 110. 2005 (Pino et al. 2005) -- Type: Peru, La Libertad, prov. Sanchez Carrión, dist. Huamachuco, Sausacocha lagoon, 7°47'24"S, 77°59'11"W, 3,290 m, *Klopfenstein & Cieza* AJABOSAM-593 (holo-: USM 184605).

The species was described as a variety of *P. macrorhiza* Kunth with similar reproductive organs and large exposed tubers growing on rocks. However, *P. lilliputiana* has much smaller leaves that are glaucous, cordate and quite succulent contrasting with the orbicular, centrally peltate leaves of *P. macrorhiza*. Phylogenetic analyses show this species to be most closely related to *P. parvifolia* (Fig. 1).

7. *Peperomia ocrosensis* G.Mathieu & Pino**, sp. nov.**

A *P. rupiseda* C.DC. differt rachidi graciliore et floris morphologia, bractea florali elliptica vel interdum parum obovate, epunctulata, ovario minute papillato, stylo glabro, stigmate stipitato tholiformi; tubere interiore aliquantum fibroso haud succulento colore pallide stramineo. -- Type: Peru, Ancash, prov. Ocros, dist. Ocros, road Paramonga--Conococha (via Ocros), km mark 29, 10°27'32.4"S, 77°24'29.6"W, 3,105 m, 3 Feb. 2009, *Mathieu et al.* 2009-042 (holo-: USM; iso: BR, GENT, K, MO).

Rather robust herb, 10--15 cm tall. Tuber globose, up to 3.5 cm diam, slightly flattened, developing irregular outgrowths from apex when becoming older, then becoming higher than wide, surface brown, slightly rough, yellowish beige on cross section, internal texture rather dry and fibrous, rooting at apex. Leaves 5--12 per plant; petiole 9--12 cm x 1.5--2.4 mm, terete, whitish green to whitish pink with red striae; lamina orbicular, to 4 cm diameter, rather firm, undulate, centrally peltate, 8(--9)-palmatinerved, nerves branched. Spadices (1--)5--12(--17) per plant; peduncle 9--11 cm x 1.4--1.6 mm, light green or with a reddish tinge; rachis 4.5--6.5 cm x 1.4--1.8 mm, light green, moderate-densely flowered. Floral bracts elliptic to sometimes slightly obovate, 0.75--0.85 x 0.5.--0.65 mm, centrally peltate, apex short acuminate, light green. Stamens with slender filaments 0.3--0.6 x 0.16--0.18 mm, perpendicular to rachis; anthers ellipsoid, 0.4--0.6 mm, white, brown and collapsed when dry. Ovary globose to broad ellipsoid, 0.3--0.4 x 0.3--0.35 mm, short pedicellate, intense green, entirely minutely papillate; style broad conical to obovoid, 0.52--0.3 x 0.3--0.35 mm, yellowish green, lighter than ovary; stigma terminal, pedicellate, dome-shaped, 0.2--0.25 mm diameter, stigmatal fimbriae white, surpassing edge of stigmatal plateau. Fruit not seen. Figs. 7, 8.

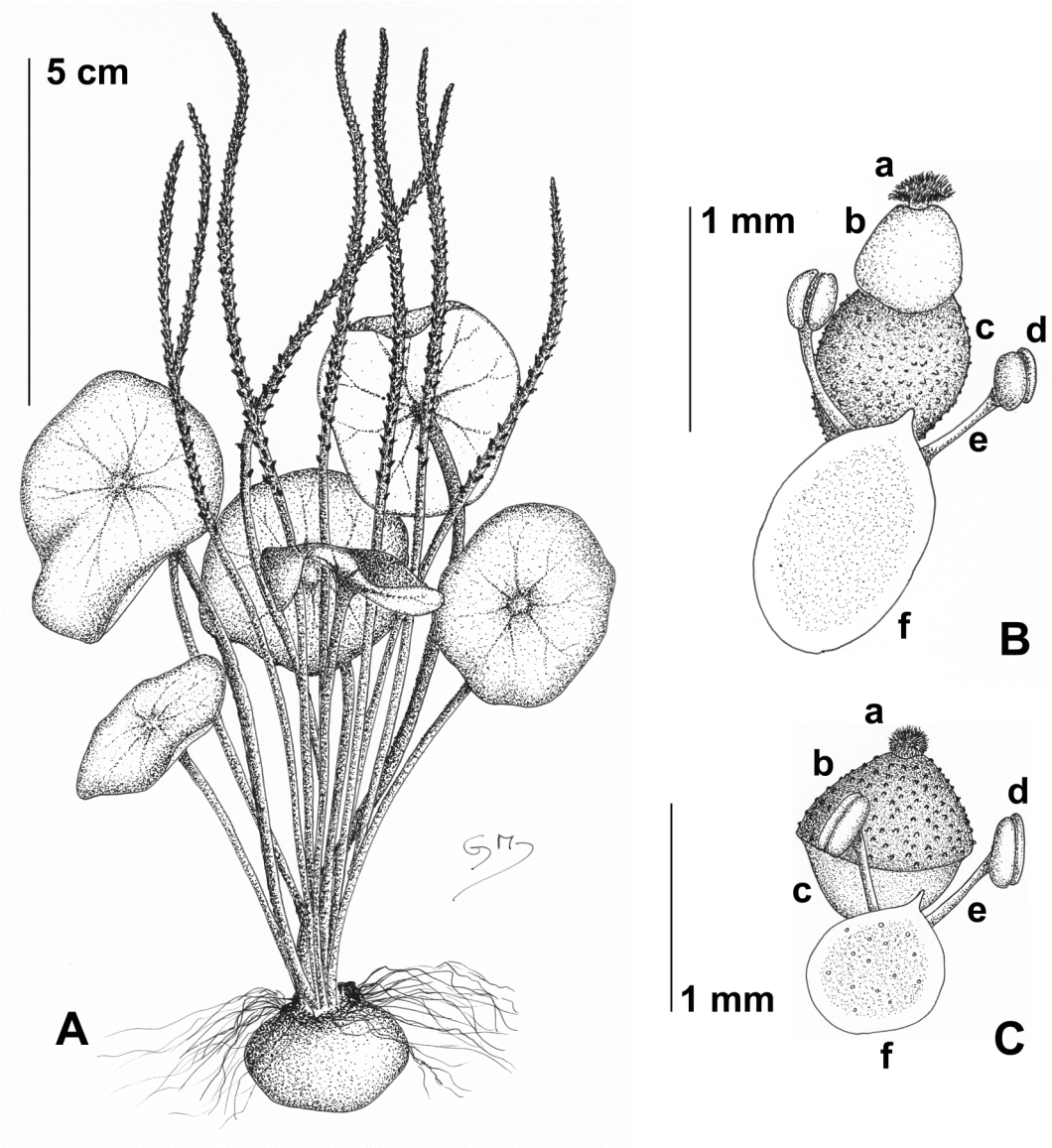


Figure 7. **A,B, *Peperomia ocrosensis*; C, *P. rupiseda*: A, general habit; B, flower; C, flower, a. stigma, b. style, c. ovary, d. anther, e. filament, f. floral bract.**

Figure 8. **Tubers on cross section: *P. ocrosensis*, young tuber (left), older tuber (middle), *P. rupiseda* (right).**

Etymology -- The specific epithet refers to Ocros, the closest village, ca 6 km N of the type locality.

Relationships -- *Peperomia ocrosensis* exhibits the robust general habit of *P. rupiseda* C.DC. Both species show rather large, slightly undulate leaves. *P. ocrosensis*, however, has much thinner inflorescences (rachis 1.5 mm diameter versus 3.5 mm in *P. rupiseda*). They also differ in the characters of the style, most clear in young carpels. In *P. ocrosensis*, the smooth style is lighter than the body of the ovary and apically positioned on the minutely papillate ovary. In *P. rupiseda* on the contrary, the minutely papillate style is darker than the ovary and dome-shaped 'over' the ovary, thus covering its apical part. *P. ocrosensis* shows a pedicellate, dome-shaped stigma that surpasses the limits of the stigmatal plateau whereas in *P. rupiseda* the stigma is sessile and globose. The tuber of *P. ocrosensis* is yellowish beige on cross section whereas in *P. rupiseda* it is creamy white with a darker brownish peripheral zone (Fig. 8). Inside, the tuber of *P. ocrosensis* is rather dry and fibrous, juicy in *P. rupiseda*. Also their root position is different (rooting from the apex in *P. ocrosensis* versus from all over the surface in *P. rupiseda*). In a phylogenetic analysis, *P. ocrosensis* is sister *P. querococha*na G.Mathieu & Pino (Fig. 1).

Phenology -- The plant has been found above-ground in February, flowering but not fruiting.

Distribution and habitat -- *Peperomia ocrosensis* is only known from its type locality in the Peruvian department of Ancash. It was found at an elevation of 3,105 m, growing in clayish soil, at the base of an exposed but overhanging rock wall.

8. *Peperomia parvisagittata* G.Mathieu & Pino, **sp. nov.**

A speciebus geophilis australo-americanis notis distincta lamina ovata vel deltoidea, cordata, vulgo non-peltata vel minute peltata. -- Type: Peru, Ancash, prov. Bolognesi, dist. Cajacay, road Huaraz--Pativilca km 103, 940 m before Majoralca, ca 250 m uphill Cerro Huansharury, in mosses on vertical sandy rock wall above *Eucalyptus* plantation, 10°09'31.4"S, 77°21'13.3"W, 3,480 m, 25 Jan. 2009, *Mathieu & Symmank* 2009-020 (holo-: USM; iso-: BR, GENT).

Delicate herb, up to 2--3.5 cm tall in vegetative stage. Tuber usually buried, up to 0.8 cm diameter, globose or slightly higher than wide, rooting at base, surface yellowish-green to light brown or reddish. Leaves 2--6(--13) per plant; petiole 1--3 cm x 0.25--0.4 mm, terete, light green, often whitish at the base; lamina chartaceous, flat or slightly folded upward along the main nerve, cordiform, ovate to deltoid or, more rarely, almost orbicular, 4--8 x 4--6 mm, apex acute to obtuse, rarely emarginate, base usually cordate, rarely rounded, usually non peltate or slightly peltate, rather dark green adaxially, lighter, whitish green abaxially, nervation obscure, usually only main nerve noticeable. Spadices (1--)2--5 per plant; peduncle 2--6 cm x 0.35--0.45 mm, light green, often whitish at the base, sparsely minutely dotted, usually twice as long as petiole; rachis 1.5--5 cm x 0.55--0.85 mm, same length or a little shorter than peduncle, towards the apex gradually becoming wider, apically up to twice as thick as peduncle, light green, more intense towards apex, sparsely minutely dotted, loosely flowered. Floral bracts orbicular to elliptic, (0.5--)0.8--0.9 x (0.3--)0.5--0.6 mm, centrally peltate, abaxially convex, apex acute to acuminate, base rounded, pedicel thick, 0.2--0.25 mm diameter. Stamens with short filaments, c 0.2 mm long; anthers 0.2--0.25 mm long, rapidly becoming brown. Ovary globose to ellipsoid, 0.25--0.30 mm diam, intense green; stigma terminal, flabelliform spherical, 0.2--0.3 mm diameter, white. Fruit ellipsoid, 0.9 x 0.6 mm, sessile, centrally attached at the base; pericarp minutely papillate; style wide conical to mammiform. Fig. 9.

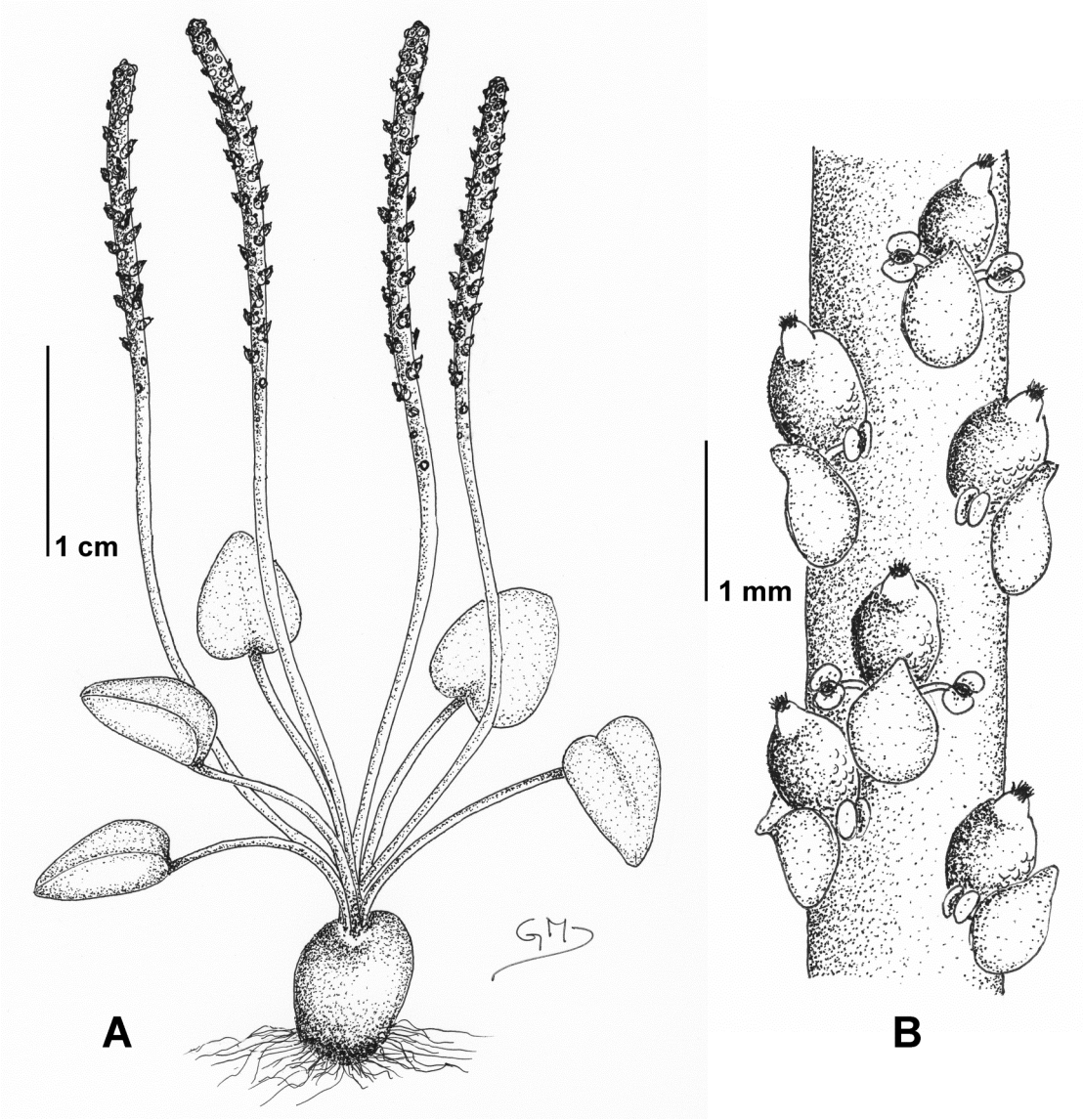


Figure 9. ***Peperomia parvisagittata*****: A, general habit; B, part of fruiting rachis.**

Other representative specimens examined -- **Bolivia:** Tarija, prov. Méndez, near Colorado Norte, 21°11'14.6"S, 64°46'18.9"W, 3,060 m, 30 Jan. 2008, *Samain et al.* 2008-083 (GENT, LPB).

Peru: Lima, prov. Canta, dist. Lachaki, Panamericana Norte, near km 95, 11°29'10.8"S, 76°38'37.6"W, 2,400 m, 17 Jan. 2009, *Samain et al.* 2009-003 (GENT, USM); prov. Huarochirí, dist. San Mateo, E slope of the Cerros de San Mateo, 3,000 m, 3 Feb. 1968, *Cerrate* 4318 (USM 67220).

Etymology -- The specific epithet refers to the leaf shape, the most characteristic feature of this species.

Relationships -- Although non-peltate leaves do occur in several Central American geophytes, *P. parvisagitta* is, together with *P. lilliputiana* (Pino & Cieza) Pino, the only South American geophytic species showing non-peltate leaves. The latter species shows succulent leaves and a large irregular tuber. Phylogenetic analysis reveals *P. parvisagittata* to be related to *P. scutellifolia* Ruiz & Pav., *P. cerrateae* Pino & G.Mathieu, *P. ayacuchoana* Pino & Samain and *P. pseudoverruculosa* G.Mathieu (Fig. 1).

Phenology -- The plant has been found above-ground in January and February, flowering and fruiting.

Distribution and habitat -- Although the species is known from only four collections, it seems to have a quite wide distribution: from the Peruvian departments of Ancash and Lima down to the department of Tarija in southern Bolivia. The elevation range covers at least 1,000 m (2,400--3,400 m), growing between mosses on vertical rock walls.

9. *Peperomia peruviana* Dahlst. (Dahlstedt 1900: 92)

Basionym rejection and lectotypification

Describing the genus *Tildenia*, Miquel included a single species: *Tildenia mexicana* Miq. (Miquel 1843a: 82). Shortly afterwards, Miquel transferred *Peperomia umbilicata* Ruiz & Pav. to the new genus but named it *Tildenia peruviana* Miq., not upholding the epithet of the basionym (Miquel 1843b: 483). There was no reason not to use the epithet *umbilicata* in *Tildenia* and therefore, *Tildenia peruviana* has to be considered as superfluous.

Subsequently, *Tildenia* was reclassified at subgeneric rank [‘sectio’] within *Peperomia*. Miquel correctly revived *Peperomia umbilicata* Ruiz & Pav. and cited *Tildenia peruviana* Miq. as its synonym (Miquel 1843c-1844: 69). Hence, it is clear that *Tildenia peruviana* Miq. ≡ *Peperomia umbilicata* Ruiz & Pav.

Dahlstedt (1900) observed correctly that the specimen at B from “St. Franco de Hay circa Lacum Titicaca”, cited by Miquel as “St. Franco de Ary circa Lacum Titicacum. Apr. 1831. Meyen” under *P. umbilicata*, could not be considered as belonging to that species. Apart from the differences in fruit morphology he mentioned, *P. umbilicata* is a coastal Peruvian species occurring below 800 m whereas the *Meyen* collection is a different species from the high Andes. Unfortunately, Dahlstedt published the new species as *Peperomia peruviana* (Miq.) Dahlst. but he made a nomenclatural mistake (Dahlstedt, 1900, p. 32) as there was no objection against the use of the epithet *peruviana*. Miquel did not introduce the name *Peperomia peruviana* and the epithet was still available in *Peperomia*. The error is not the choice of the epithet but the citation of the basionym. If

Dahlstedt wanted to distinguish *P. peruviana* from *P. umbilicata* he could not, at the same time, refer to *Tildenia peruviana* (superfluous for *P. umbilicata*) as the basionym. This error is corrected herewith according to ICBN art. 48.1 “when an author who adopts a name refers to an apparent basionym but explicitly excludes its type, a new name is considered to have been published that must be attributed solely to that author” (McNeill et al. 2006).

Lectotype designation

In his protologue Dahlstedt (1900) refers to five collections of which *Meyen’s* specimen at B is mentioned first. That specimen was destroyed during World War II and a lectotype has to be chosen from other material cited. Also mentioned is *Gaudichaud 150* at G-DC, but that collection (G, G-DC, MO, NY, P [3], U) has to be excluded. In 1936 it became the type of *Peperomia hillii* Trel., a synonym of *P. umbilicata*. Dahlstedt already cited it under the latter name (l.c., p. 31), although it was included at the same time under *P. peruviana*. Neither the B nor the Hb Gris. (currently GOET) specimen of *Lorentz & Hieronymus, Fl. arg. no 658* could be found. Of the two remaining collections, *Lorentz & Hieronymus 2* and *Schickendantz 165*, a specimen is extant at GOET. Both specimens bear the annotation: “det. H. Dahlstedt 1901”. *Schickendantz* 165 is chosen as the lectotype because it best exhibits the morphology of the species and mentions a precise collection site.

Although a lectotype is designated herewith, the locality of the lost Meyen type is still important in the view of future collections that might provide support for the current *P. peruviana* concept. The locality “St. Franco de Hay” (by Dahlstedt) or “St. Franco de Ary” (by Miquel) has puzzled many botanists. The difference in spelling suggests that the writing of the original label might have been obscure. We assume that it was actually meant “St. Franco de Anq.”, standing for San Francisco de Anquac, already in 1831 an abandoned mission station, about 2½ hours walking distance from Lake Titicaca and 3 hours W of the small town of Jull [apparently Juli]. In his travel reports, Meyen pointed to a series of conspicuous parallel rock walls, running in SW-NE direction, 20--30 feet high and 4--5 feet wide, just before reaching the mission station [coming from the south] on April 7th and he especially mentioned the many small but very attractive plants that where growing on these walls (Meyen 1834: 478). This is very likely a direct reference to *P. peruviana* and the precise location where it was collected.

10. *Peperomia pseudoperuviana* (Pino) Pino, **stat. nov.**

Peperomia andina var. *pseudoperuviana* Pino, Peperomias de Cajamarca: 14. 2004. (Pino 2004). -- Type: Peru, Cajamarca, prov. Cajamarca, dist. San Juan, road Cajamarca--San Juan km 146, in crevices on rock wall, in mosses, together with *Oxalis* sp., *Peperomia cymbifolia* Pino and *Peperomia galioides* Kunth (Pino 2010), 7°17'32"S, 78°29'27"W, 2,370 m, 4 Oct. 2002, *Pino* 894 (holo-: USM 184602).

The species was first described as a variety of *P. andina* but it proved to have several morphological differences. It grows in the same region although it prefers lower elevations and more sheltered places in the shade. Distinct are the orbicular and flat leaves, peltate in the middle, whereas *P. andina* shows larger, oval or subtriangular funnel-shaped leaves. The tubers of *P. pseudoperuviana*, usually buried under moss, remain quite small.

Other representative specimens examined -- **Peru**: Cajamarca, prov. Cajamarca, dist. San Juan, Road Cajamarca--San Juan km. 148.5, between rocks, 7º15'58"S, 78º30'39" W, 2660 m, 3 May 2002, *Pino* 298 (USM); road San Juan--Huacraruco, on rock wall, 7º17'35"S , 78º29'18" W, 2400 m, 16 May 2000, *Pino* 642 (USM).

11. *Peperomia pseudoverruculosa* G.Mathieu, **sp. nov.**

A *P. verruculosa* Dahlst. ex Hill differt tubere ex apice radicanti, spadicibus longioribus et laminis orbicularibus; a *P. ayacuchoana* Pino & Samain differt tubere minore, foliis majoribus et fructibus verruculosis. -- Type: Peru, Ayacucho, prov. Huamanga, dist. Acocro, road Ayacucho--Chincheros, km 62, 13°18'55.7"S, 73°58'48.8"W, 3,450 m, 26 Jan. 2009, *Samain et al.* 2009-052 (holo-: USM; iso-: BR, GENT).

Small herb, up to 10 cm tall. Tuber globose, up to 1 cm diameter, forming apical outgrowth half the size of tuber when growing older, surface smooth, yellowish to light brown rooting at apex. Leaves (3--)5--13 per plant; petiole (1.5--)3--5(--7) cm x 1 mm, terete, whitish translucent green, sometimes slightly tinged pink-purple, often whitish at base; lamina membranous but not translucent except for peripheral 0.2 mm, orbicular, (0.5--)0.7--1.0(1.2) cm diameter, apex often minutely emarginate, flat but centrally slightly depressed, centrally peltate, vivid green adaxially, dull whitish green abaxially, nervation obscure, only main nerve distinct, especially in dried specimens, slightly protruding abaxially, slightly depressed adaxially, minutely yellow-brown glandular-dotted abaxially when dried. Spadices 4--12 per plant; peduncle same length as petiole or a little longer, 4--6(--8) cm x 1 mm, whitish at base, becoming green more apically; rachis same length or

longer than peduncle, up to 8 cm x 1.5 mm, about same diameter as peduncle, usually slightly darker green than peduncle, loosely flowered. Floral bracts elliptic, 0.7--0.8 x 0.5--0.6 mm, apex acuminate, base rounded, centrally peltate, succulent and a little convex adaxially, margin membranous. Stamens with short filaments, 0.1--0.2 mm long; anthers ca 0.3 mm long. Ovary ellipsoid; surface granulose; style ovoid to mammiform, larger than ovary in young carpels; stigma terminal, dome-shaped or a little flatter, stigmatal fimbriae surpassing the edges of stigmatal plateau. Fruit ovoid, body 0.7 x 0.6 mm, sessile and slightly sunken in rachial pit, centrally attached at the base; pericarp polygonal verruculose; style short conical to mammiform, 0.2--0.3 mm long. Fig. 10.

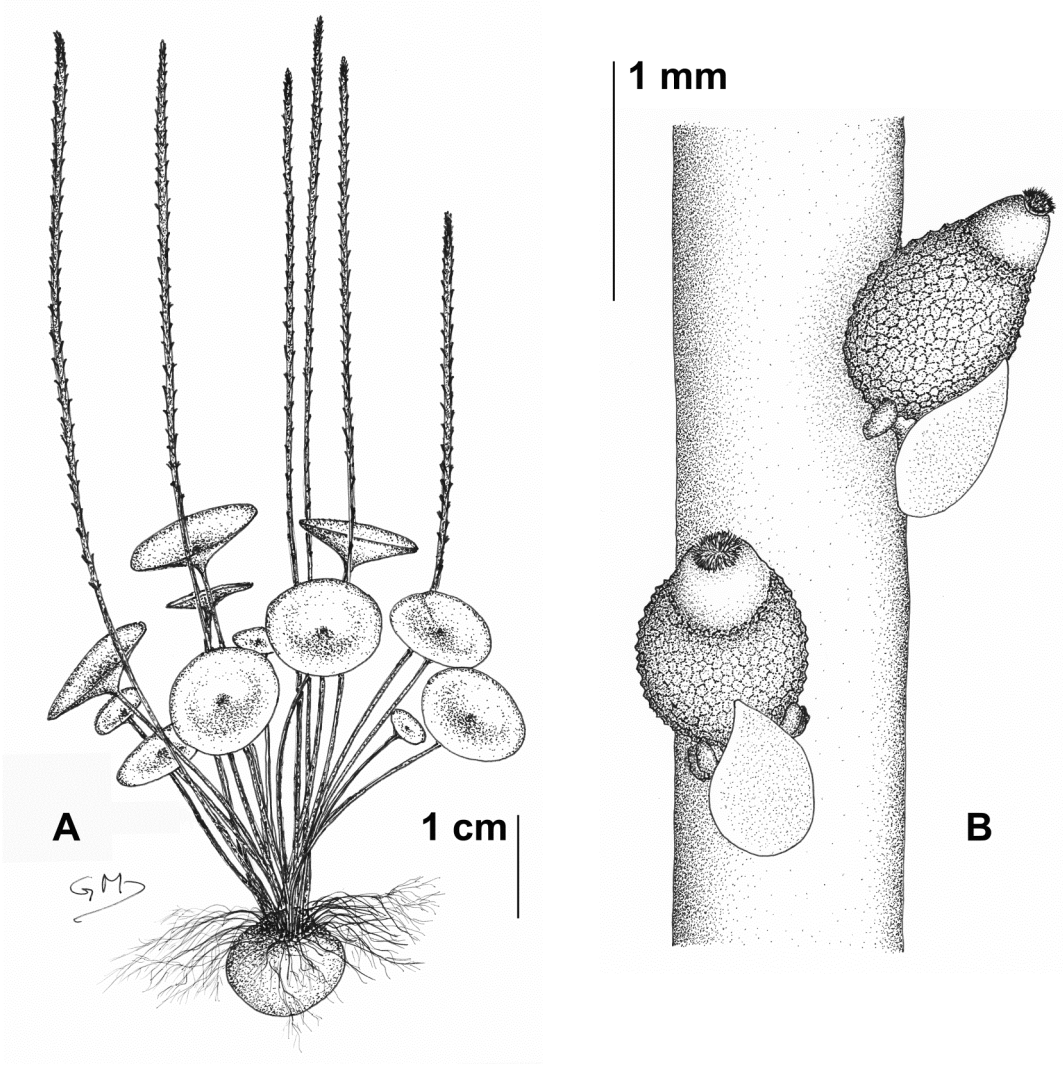


Figure 10. ***Peperomia pseudoverruculosa*: A, general habit; B, part of fruiting rachis.**

Other representative specimens examined -- **Peru**: Apurimac, prov. Chincheros, dist. Ocobamba, along road Chincheros--Abancay, km 165, 13°32'22.3"S, 73°40'24.2"W, 3,290 m, 26 Jan. 2009, *Samain et al.* 2009-061 (GENT, USM); loc. cit., km 175, 13°32'48.4"S, 73°37'43.2"W, 3,760 m, 27 Jan. 2009, *Samain et al.* 2009-062 (BR, GENT, USM); prov. Andahuaylas, dist. Huancarama, along road Chincheros--Abancay, km 328, 13°42'01.3"S, 73°02'47.4"W, 3,470 m, 27 Jan. 2009, *Samain et al.* 2009-072 (GENT, USM); Ayacucho, prov. Huamanga, dist. Quinua, along the "Via Los Libertadores" Pisco--Ayacucho, 13°16'52.3"S, 74°18'25.7"W, 3,730 m, 23 Jan. 2009, *Samain et al.* 2009-036 (BR, GENT, USM); Cuzco, prov. Cuzco, dist. Cuzco, 6 km from San Jerónimo Huaccoto, 13°31'05.5"S, 71°52'14.0"W, 3,890 m, 13 Feb. 2009, *Samain et al.* 2009-122 (BR, GENT, USM); behind the ruins of Sacsayhuamán, near the road Cuzco--Calca km 7--8, 13°30'08.3"S, 71°58'48.2"W, 3,660 m, 13 Feb. 2009, *Samain et al.* 2009-123 (BR, GENT, USM); along the road Cuzco--Calca km 8--9, 13°29'47.8"S, 71°58'24.1"W, 3,740 m, 13 Feb. 2009. *Samain et al.* 2009-124 [fruits with gals] (BR, GENT, USM).

Etymology -- The specific epithet refers to the verruculose fruit pericarp. In this respect, the species resembles *P. verruculosa* Dahlst. ex Hill.

Relationships -- *Peperomia pseudoverruculosa* can be distinguished from the three other Andean species with fruits with a verruculose pericarp (*P. verruculosa*, *P. parvifolia* C.DC. and *P. pugnicaudex* Pino). These three species have tubers rooting at the base whereas the tuber of *P. pseudoverruculosa* roots at the apex. The Bolivian *Peperomia radiatinervosa* G.Mathieu also shows verruculose fruits and a tuber rooting from the apex but this species has much larger, distinctly nerved leaves.

At its type locality, *P. pseudoverruculosa* grows together with *P. verruculosa* and both species apparently prefer the same habitat. However, *P. pseudoverruculosa* can easily be distinguished morphologically by the tuber (lighter colour, smooth surface, apical root position), the longer inflorescences and the orbicular leaves. Its floral bracts show a more acute to acuminate apex and are more slenderly stalked. Finally, the fruits do not show the almost discoid style of *P. verruculosa*. According to phylogenetic analysis, *P. pseudoverruculosa* is not closely related to any of the species mentioned. Its closest relative appears to be *P. ayacuchoana* Pino & Samain (Fig. 1).

Phenology -- The plant has been found above-ground in January and February, flowering and fruiting.

Distribution and habitat -- *P. pseudoverruculosa* occurs in the Peruvian departments of Apurimac, Ayacucho and Cuzco, at rather high elevations (3,000--4,000 m) on eastern Andean slopes. It prefers an exposed, grassy and shrubby habitat where it grows in soil pockets and crevices between and on rocks.

12. *Peperomia pugnicaudex* Pino, **sp. nov.**

Planta geophila tubere epigaeo basi radicibus instructo; caudex expositus, globosus vel late infundibuliformis, obscure lobatus; folia petiolata sub medio peltata, lamina rotundata coriacea 1.2--1.8 cm diam, supra concava, subtus purpurascens, marginibus saepe undulatis; a *P. chutanka* Pino differt caudice crassiore brevioreque, foliis minoribus, spadicibus brevioribus et baccis ovoideis verruculosis. -- Type: Peru, Lima, prov. Huarochirí, dist. San Mateo, central highway km 98, beyond Infiernillo railroad bridge, S bank of Rimac river, before Cacray tunnel, in front of waterfall, 11º44'45"S, 76º17'35"W, 3,300 m, 24 Feb. 2002, *Pino* 792 (holo-: USM 230319).

Medium sized herb, 7--15 cm tall. Tuber exposed, globose when young, then funnel-shaped, branching after each flowering, light brown to grayish, rooting at base, 1.5--3 cm diam at base, 6--7 cm diameter at apex, 4--5 cm tall, with knuckle-like projections on top, 0.8--1.5 cm diam. Leaves up to 50 or more per plant, 12--15 from each projection; petiole 6--12 cm x 1--1.3 mm, terete, light green with reddish longitudinal lines when exposed, straight to slightly curved; lamina orbicular, 1.2--1.8 cm diameter, coriaceous, peltate slightly below middle, base and apex rounded, margin entire, induplicate or undulate in young leaves, flat to sometimes revolute in older leaves, adaxially flat to concave, umbilicate at petiole insertion, dark green, nerves invisible, abaxially funnel-shaped, reddish to purplish when more exposed, 7--9-palmatinerved, nerves darker red or purple. Spadices 3--6 from each tuberous projection, erect or slightly curved with upward apices; peduncle 1.2--3 cm x 1--1.4 mm, whitish or very light green; rachis 1.5--3 cm x 1.3--1.6 mm, light green. Floral bracts peltate, ovate, slightly more red than rachis, 0.8 x 0.6 mm. Stamens with short filaments; anthers globose, 0.4--0.5 mm diameter, white, drying brownish gray. Ovary 0.6--0.7 x 0.4--0.5 mm, ovoid, bright green. Fruit ovoid, 1--1.1 x 0.7--0.8 mm; pericarp minutely verruculose, reddish brown; style widely conical, bright green. Fig. 11.

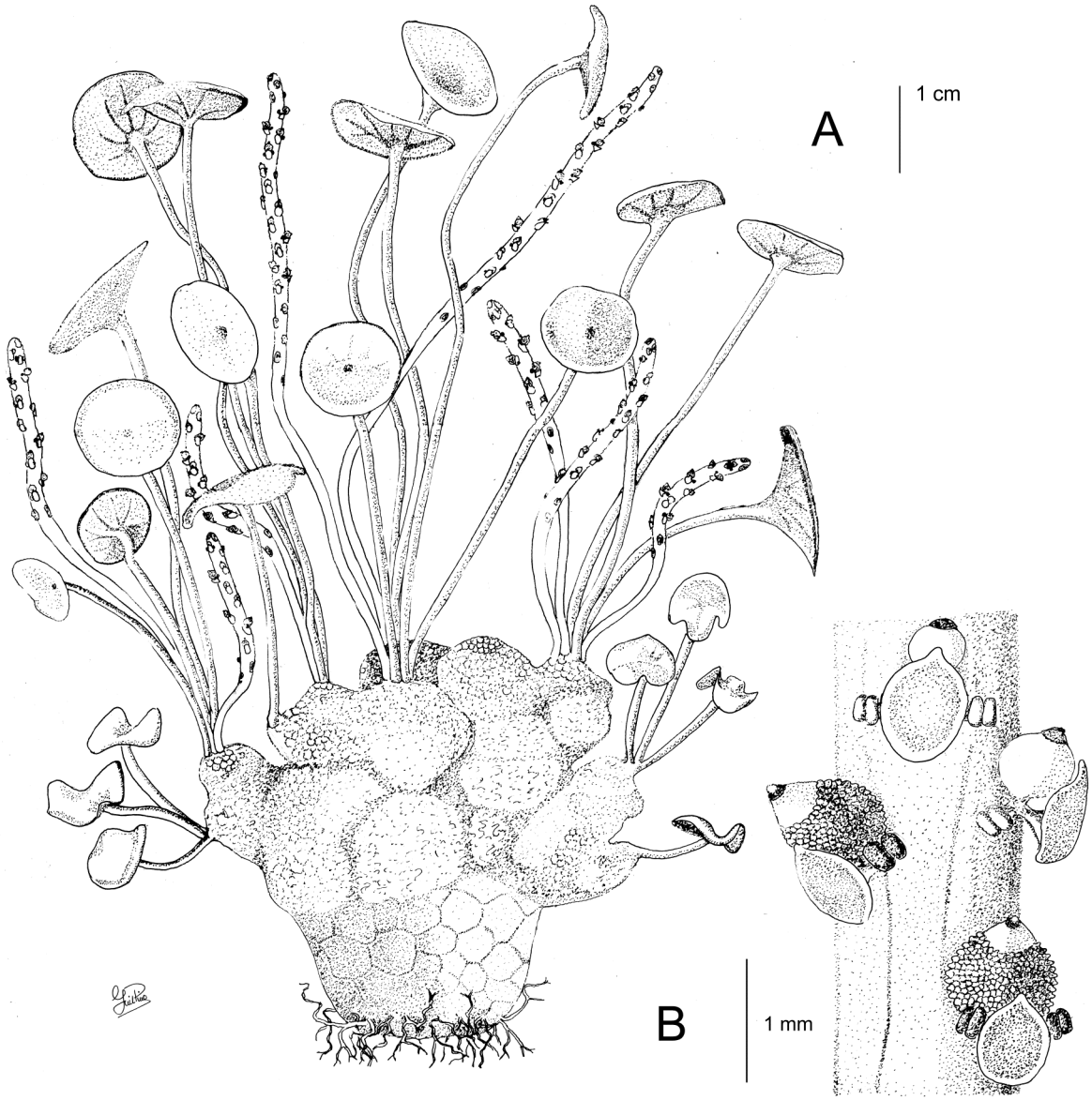


Figure 11. ***Peperomia pugnicaudex*: A, general habit; B, part of rachis.**

Other representative specimens examined -- **Peru**: Lima, prov. Huarochirí, dist. San Mateo, central highway km 98, beyond Infiernillo railroad bridge, N bank of Rimac river before waterfall, 11º44'24"S, 76º16'49"W, 3,430 m, 18 Jan. 2009, *Samain et al.* 2009-007 (GENT, USM).

Etymology -- The aerial tuber parts of this species look like a closed hand, "pugnus" = fist in Latin.

Relationships -- The tubers of *P. pugnicaudex* produce irregularly branching outgrowths only comparable with those of *P. chutanka* Pino, with which it shares the vernacular name 'chutanka'. However, tubers in *P. pugnicaudex* are smaller and thicker, and definitely do not look like stems like in *P. chutanka*. The leaves are reddish, coriaceous and funnel-shaped like they are in *P. chutanka*, but they are much smaller, constantly orbicular and with more undulate margins. Spadices are more abundant but shorter and fruits are ovoid and verruculose compared to the odd cylindrical drupes of *P. chutanka*. According to molecular data this species is most closely related to the clade consisting of *P. ocrosensis* G. Mathieu & Pino and *P. querocochana* G.Mathieu & Pino (Fig. 1).

Phenology -- Leaves are present from December to April, spadices appear from October to January and fruits ripen from December to February.

Distribution and habitat -- It grows in clayish soil among rocks, in half shaded places. *P. pugnicaudex* appears to be rare and very few specimens have been found at the only known locality. Careful efforts have to be made for its conservation.

13. *Peperomia purpureonervosa* G.Mathieu, **sp. nov.**

A speciebus geophilis notis distincta nervis laminae, supra reticulatis atro-viridibus, infra purpureis. -- Type: Bolivia, La Paz, prov. Nor Yungas, road Unduavi--La Paz, 2.7 km from La Paz, on rocks along the road, shaded by shrubs, 16°18'54.8"S, 67°55'28.4"W, 3,270 m, 20 Jan. 2008, *Symmank et al.* 2008-051 (holo-: BR; iso-: GENT, LPB, MO).

Delicate herb, usually not more then 5--8 cm tall due to decumbent petioles. Tuber globose when young, ca 1 cm diameter, developing apical outgrowths and becoming irregular when growing older, rooting all over its surface, dark brown to almost black, creamy white on cross section with a rather thick peripheral dark brown zone. Leaves 2--8 per plant; petiole 5--11 cm long, whitish near base, rest purplish, longitudinally striate distally; lamina membranous, translucent, slightly irregularly orbicular, 1--4 cm diameter, apex rounded or often emarginate, centrally peltate, (4--5--) 6-palmatinerved, main nerves profusely branching and anastomosing near margin, reticulately nerved in between, nerves and small zone along them usually distinctly contrasting with remainder of lamina, dark green on light green background adaxially, purple (or slightly magenta) on whitish green or purplish tinged background abaxially, umbilicus yellowish with dark centre. Spadices 1--6 per plant, filiform; peduncle 2--12 cm long, whitish green to purplish, longitudinally striate; rachis usually longer than peduncle, except in young spadices, about same diameter as peduncle, whitish green to purplish, longitudinally striate, very loosely

flowered, individual flowers up to 3 mm apart. Floral bracts short elliptic, 0.7 x 0.5 mm, centrally peltate, apex acuminate. Stamens with filaments 0.4 mm long; anthers 0.2 x 0.15 mm. Ovary ellipsoid, 0.4 mm long; stigma terminal. Fruit long ellipsoid, body 0.8 x 0.5 mm; shortly pedicellate; pericarp granulose; style long conical, 0.4 mm. Figs. 12, 13.

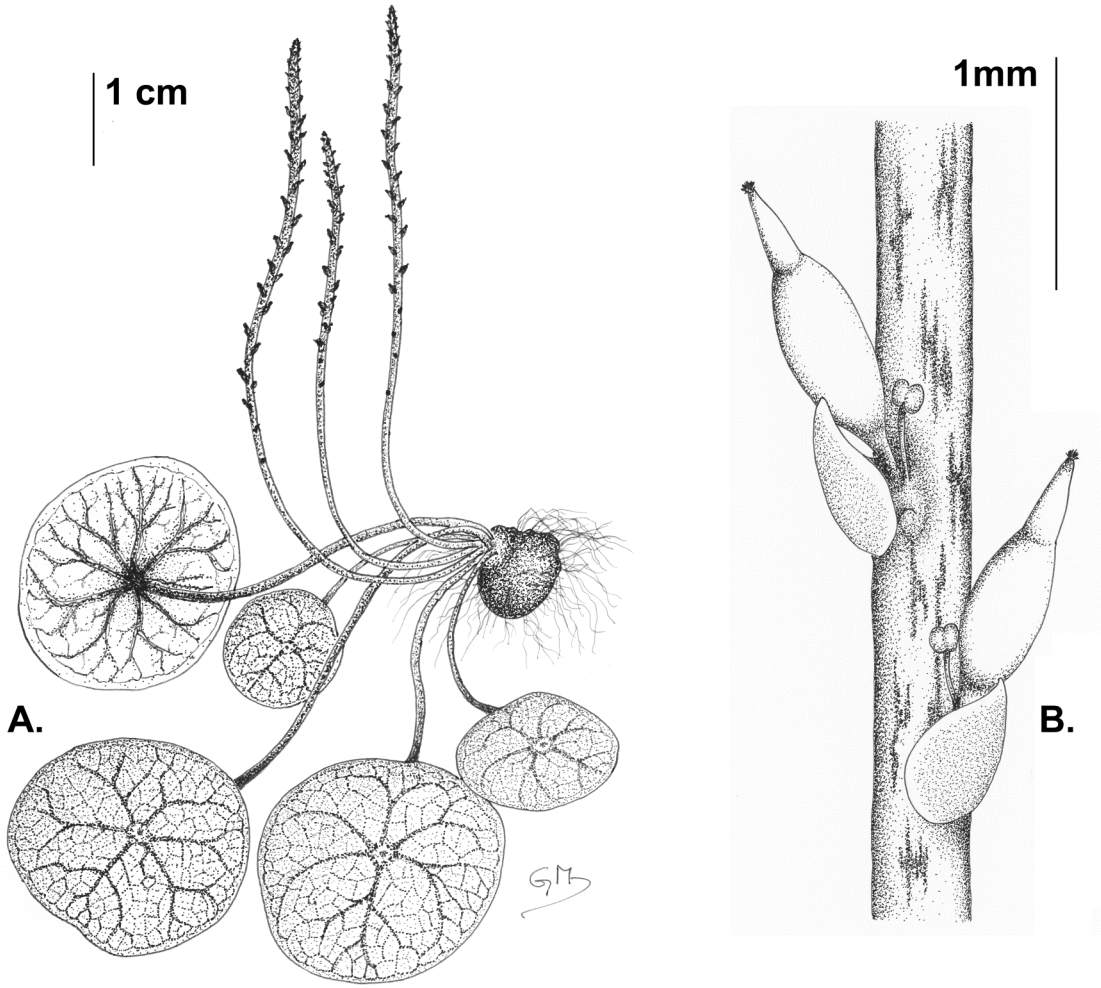


Figure 12. ***Peperomia purpureonervosa*: A, general habit; B, part of rachis.**

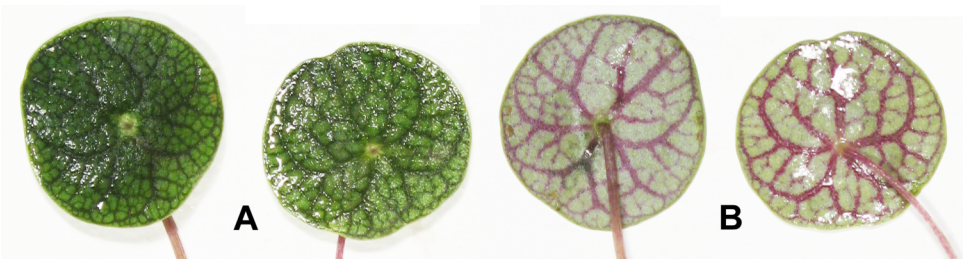


Figure 13. ***Peperomia purpureonervosa*, leaf nervation (pattern and colour): A, adaxially; B, abaxially.**

Other representative specimens examined -- **Bolivia**: La Paz, prov. Franz Tamayo, Integrated Management Natural Area (ANMI) Apolobamba, sector Laitiki--Piara, between Pelechuco and Apolo, saxiculous herb in shade, spadices purple-red, 14°47'01"S, 69°01'08"W, 2,659 m, 20 Apr. 2006, *Fuentes et al.* 10409 (BR, LPB, MO)

Etymology -- The specific epithet refers to the purple colour at and along the nerves abaxially.

Relationships -- Phylogenetically *P. purpureonervosa* appears to be related to *P. peruviana* Dahlst. (Fig. 1). Morphologically *P. purpureonervosa* is quite distinct in the colour at and along the nerves (dark green adaxially, purple abaxially) (Fig. 13), which is, due to the translucent leaves, also clearly visible in the herbarium specimens. However, as it is evident from *Fuentes 10409 et al.*, the purple colour may not be that prominently present in every population or can even lack completely in individual specimens. In those cases, the reticulate nervation pattern remains diagnostic as the secondary nerves in the leaves of *P. peruviana* are nearly invisible. The leaves of *P. purpureonervosa* can be twice as wide as in *P. peruviana* and are also more translucent.

Phenology -- The plant is above-ground at least from January to April, flowering and fruiting.

Distribution and habitat -- Growing in moss on rock walls in the Bolivian department of La Paz.

14. *Peperomia querococha*na G.Mathieu & Pino, **sp. nov.**

A *P. umbrosa* G.Mathieu differt lamina distincte nervosa et tubere e basi radicanti, parte basali laevi flavaque, parte apicali aspera brunneaque, ramificanti ubi vetusto. -- Type: Peru, Ancash, prov. Recuay, dist. Recuay, Cordillera Blanca, road Ticapampa--Chavin de Huántar, near Lake Querococha, along brooklet uphill from road, in mosses, 09°43'40.2"S, 77°19'40.5"W, 4,100 m, 22 Jan. 2009, *Mathieu & Symmank* 2009-017a (holo-: USM; iso-: BR, GENT, MO).

Delicate herb, 8--12 cm tall. Tuber globose, surface entirely smooth and yellowish when young, gradually developing apically brown and rough zone, distinctly separated from remaining yellowish basal part, finally completely replacing yellowish part, loosing globose aspect when becoming older by forming two or more thick outgrowths, apically bearing buds from which leaves and inflorescences develop, rooting at base, creamy

white to yellowish on cross section. Leaves 2--12 per plant; petiole slender, 5--10(--11) x 0.05 cm, white or whitish-green; lamina membranous, orbicular, (0.5--) 0.8--1.3(--2) cm diameter, flat or sometimes slightly funnel-shaped in centre, peltate in middle or slightly below, base sometimes slightly truncate, apex often widely emarginate with very tip minutely acuminate, distinctly nerved abaxially, 5--7-palmatinerved, main nerves distally anastomosing by perimarginal nerve, main nerve often slightly protruding abaxially. Spadices 1--4 per plant; peduncle 5--10 cm, white or whitish-green; rachis 1.5--2 cm x 1.2--1.5 mm, 2 times as thick as peduncle, loosely flowered, green, minutely dark dotted. Floral bracts elliptic, 1--1.1 x 0.5--0.7 mm, centrally peltate, apex acuminate to long acuminate, intense green. Stamens with filaments 0.3--0.5 x 0.08--0.09 mm, perpendicular to rachis; anthers subglobose, 0.4 x 0.3 mm, white, brown when dry. Ovary ellipsoid, 0.6--0.65 x 0.5--0.55 mm, intense green; stigma terminal, subglobose, fimbriate, 0.3--0.4 mm diam, white. Fruit ellipsoid, 0.9--1 x 0.6--0.7 mm, sessile, centrally attached at the base; pericarp granulose; style conical, slightly asymmetrical, reaching further towards base abaxially than adaxially (best visible in younger fruits), 0.2--0.25(--0.4) mm long, 0.35--0.45 mm wide, brownish. Figs. 14, 15.

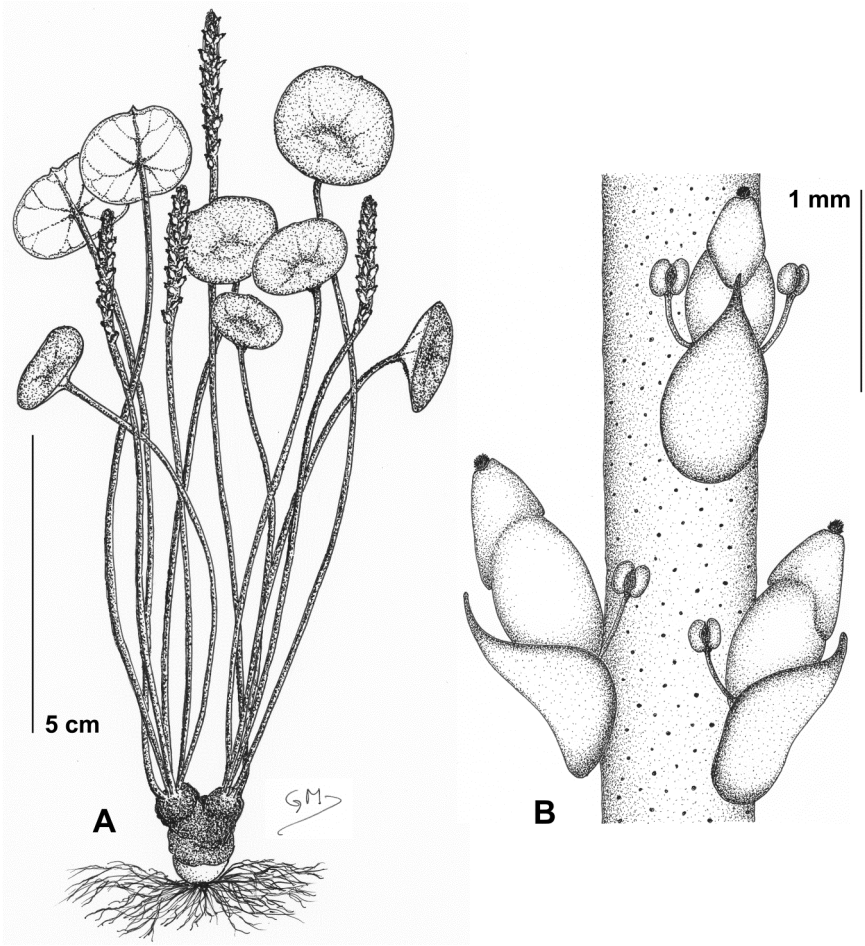


Figure 14. ***Peperomia querocochana*: A, general habit; B, part of flowering rachis.**

Figure 15. ***Peperomia querocochana*: tubers of different ages and developmental stages (young to the left, older to the right).**

Other representative specimens examined -- **Peru**: Ancash, prov. Ocros, dist. Ocros, road Ocros--Cerro Chunto km 15.5, rocky slope, ca 200 m uphill from road, 10°20'08.1"S, 77°20'88.0"W, 3,955 m, 3 Feb. 2009, *Mathieu et al.* 2009-043 (BR, GENT, USM).

Etymology -- The specific epithet refers to the locality were the type was found, near lake Querococha.

Relationships -- The species resembles *P. umbrosa* to some extent. However, the latter species has obscurely nerved leaves and a tuber rooting all over its surface. That species is also lacking the particular tuber development observed in *P. querocochana* (Fig. 15). According to phylogenetic analysis, this species is most closely related to *P. ocrosensis* G. Mathieu & Pino (Fig. 1).

Phenology -- The plant has been found above-ground in January and February, flowering and fruiting.

Distribution and habitat -- Both known collections were made near 4,000 m. Therefore, it seems that the species is restricted to rather high locations.

15. *Peperomia radiatinervosa* G.Mathieu, **sp. nov.**

A *P. polycephala* Trel. differt tubere ex apice radicanti, lamina leviter undulata infundibuliformique, petiolo longiore, spadice robustiore, bacca majore sed bractea florali minore, crescit in saxis gramineis nec rupibus muscosis. -- Type: Bolivia, Chuquisaca, prov. Oropesa, hillside along road Sucre--Tarabuco, 8 km from Sucre, together with grasses in rock crevices, 19°05'11.2"S, 65°13'14.5"W, 2,900 m, 3 Feb. 2008, *Samain et al.* 2008-090 (holo-: BR ; iso-: G, GENT, K, LPB, MO).

Rather robust herb, 10--20 cm tall. Tuber flattened globose or slightly irregular, to 4 cm diameter but usually smaller, forming apical outgrowths when growing older, surface dark brown, rather smooth except for the outgrowths, rooting at apex, sometimes a few lateral roots. Leaves 5--12 per plant; petiole (5--)10--12(--20) cm x 2--2.5 mm, terete, whitish at the base and pinkish purple more apically, or entirely purple; lamina orbicular or irregularly orbicular, 2--4 cm diameter, centrally peltate, usually undulate and centrally slightly funnel-shaped, apex rarely obtuse, usually rounded and exhibiting a very discrete, acuminate, whitish or yellowish tip, chartaceous, shiny green adaxially, dull whitish green or purple tinged abaxially, distinctly 6-palmatinerved, main nerves branching, almost reaching margin, adaxially protruding and usually lighter than remainder of lamina. Spadices (1--)3--10 per plant; peduncle usually a little shorter and thinner than petiole, rachis a little shorter than peduncle, to 10 cm x 2 mm, moderate-densely flowered. Floral bracts orbicular to ovate, 0.7--0.8 x 0.5--0.6 mm, fleshy, adaxially concave, apex acuminate, ca 0.5 mm distant from rachis on firm stalk. Stamens minute, filaments c 0.3 mm long; anthers 0.2 mm long. Ovary ellipsoid; surface granulose; style prominent, globose to dome-shaped, larger than the ovary; stigma globose to dome-shaped. Fruit large, fruit body broadly ellipsoid, ca 1.4 x 1.1 mm; pericarp verruculose, centrally attached at base, base somewhat sunken in rachis; style flat dome-shaped, 0.2 mm high; stigma terminal, sessile or slightly sunken, dome-shaped. Fig. 16.

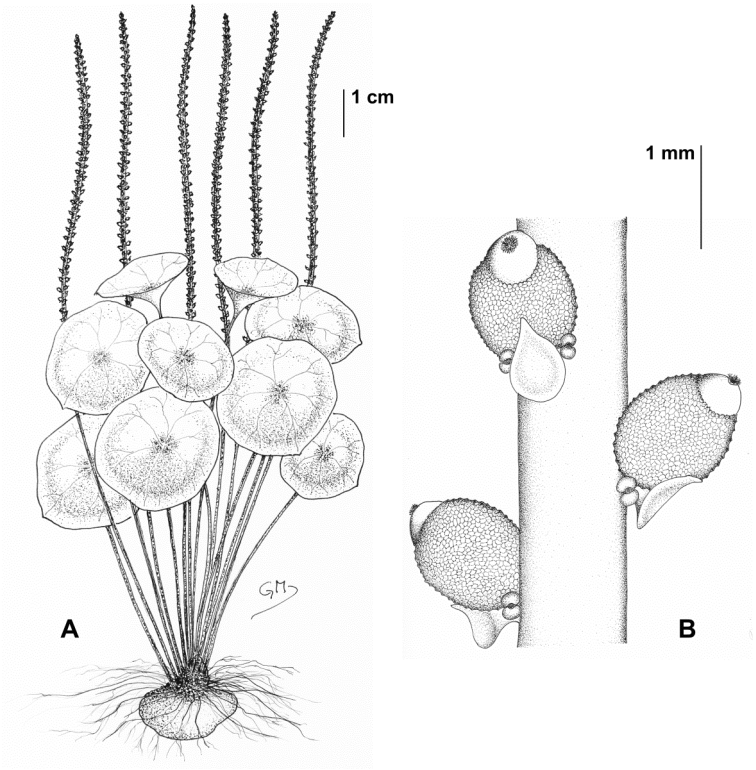


Figure 16. ***Peperomia radiatinervosa*: A, general habit; B, part of fruiting rachis.**

Other representative specimens examined -- **Bolivia**: Chuquisaca, prov. Zudañez, Mojocoya, near Buenavista, in thick humus layer, together with grasses and sedges, 18°42'14.1"S, 64°41'41.5"W, 2,535 m, 4 Feb. 2008, *Samain et al.* 2008-101 (BR, GENT, MO); loc. cit., together with mosses and grasses in rock crevices, dry habitat, 18°41'36.3"S, 64°41'37.3"W, 2,670 m, 4 Feb. 2008, *Samain et al.* 2008-105A (BR, GENT); near Villa Redencio Pampa, together with ferns, grasses, mosses and Cactaceae in rock crevices below cliff with *Puya*, 18°49'43.6"S, 64°34'57.2"W, 2,625 m, 4 Feb. 2008, *Samain et al.* 2008-110 (BR, GENT, LPB); Cochabamba, prov. Cervado, Cochabamba, Cerro San Pedro, along path from El Cristo to the Botanical Garden, steep rocky slope with *Puya* near the top of the hill, 17°24'03.3"S, 65°48'24.9"W, 3,180 m, 15 Jan. 2008, *Samain & Bates* 2008-014 (BR, GENT, LPB, MO); prov. Arani, hillside along old road Cochabamba--Santa Cruz, 6 km W of Monte Punku, together with mosses in thin soil layer between *Puya* sp. and small rocks, 17°33'35.6"S, 65°20'25.4"W, 3,030 m, 17 Jan. 2008, *Samain & Bates* 2008-028 (BR, GENT, LPB); prov. Arani, hillside along old road Cochabamba--Santa Cruz, km 328, 17°44'38.2"S 64°58'33.4"W, 3,015 m, 17 Jan. 2008, *Samain & Bates* 2008-029 (BR, GENT, LPB); prov. Campero, along road Aiquile--Santiago, together with grasses, mosses and *Puya* in shallow clayish soil in rocky area, 18°11'46.3"S, 65°16'14.0"W, 2,890 m, 9 Feb. 2008, *Samain et al.* 2008-127 (BR, GENT).

Etymology -- The specific epithet refers to the usually distinct main nerves radiating from the petiole insertion. The palmatinerved pattern is always present in the orbicular leaved species of subgenus *Tildenia* but often the nervation is obscure or the nerves are only distinct to halfway between the petiole insertion and the margin.

Relationships -- Phylogenetic analysis reveals *P. radiatinervosa* to be related to *P. cyclaminoides* Kunth and *P. verruculosa* Dahlst. ex Hill., both distinctly smaller species with ovate, not centrally peltate leaves (Fig. 1). Morphologically *P. radiatinervosa* is closer to the Peruvian *P. polycephala* Trel. Although the leaves of *P. polycephala* can reach the same diameter, the usually shorter petioles, (2.5--)3--5(--7) cm, make this species appear smaller. Leaves of *P. polycephala* are usually not undulate, flat instead of slightly funnel-shaped and thinner. The leaf apex is rounded without a minute acuminate tip. Spadices of *P. polycephala* are more slender but usually conspicuously long, 3--4(--5) times as long as the petioles. The tubers of *P. polycephala* are not larger than 10--15 mm and root from all over their surface. This species also has smaller fruits but larger floral bracts. *P. polycephala* occurs at a similar elevation but prefers a more humid habitat. It is observed on rock walls, growing together with mosses.

Phenology -- The plant has been found above-ground in January and February, flowering and fruiting.

Distribution and habitat -- *Peperomia radiatinervosa* is apparently abundant in dry rocky and grassy habitats at an elevation of 2500--3200 m.

16. *Peperomia umbrosa* G.Mathieu, **sp. nov.**

A *P. cerrateae* Pino & G.Mathieu differt tubere, e superficie tota radicanti, aspera brunneaque; a *P. querococha*na G.Mathieu & Pino differt tubere, e superficie tota radicanti, lamina obscuro nervosa. -- Type: Peru, Cajamarca, prov. Cajamarca, dist. Cajamarca, Cumbemayo, ca 20 km SW of Cajamarca, trail along (partly) underground creek, 07°11'29.7"S, 78°34'48.0"W, 3,560 m, 18 Feb. 2009, *Mathieu et al.* 2009-122 (holo-: USM; iso-: BR, GENT).

Delicate herb, up to 8 cm but often not more than 3--5 cm tall due to decumbent petioles and peduncles. Tuber 0.6--0.8 cm diameter, globose or slightly higher than wide when growing older, rooting all over its surface but mainly at the base, surface dark brown, rough, creamy white on cross section with distinct brown peripheral zone. Leaves 3--6 per plant; petiole terete, filiform, up to 12 cm long, whitish, uncommonly slightly tinged pink-red; lamina membranous, orbicular, 0.8--1.5 cm diameter, sometimes slightly emarginate, centrally peltate, vivid green adaxially, whitish green abaxially, obscurely 6-palmatinerved, nerves only visible abaxially. Spadices (1--)2--5 per plant; peduncle up to 9 cm long, shorter than petiole, whitish, rarely slightly tinged pink-red; rachis 1.5--2.5 cm long, 1.5 times as thick as peduncle, loosely flowered, whitish green, little darker apically. Floral bracts elliptic, 0.7--0.8 mm long, apex acute to acuminate, base rounded, centrally peltate, translucent, minutely vividly glandular-dotted. Stamens with filaments 0.3 mm long; anthers 0.2 mm long. Ovary shortly ellipsoid, stigma terminal. Fruit shortly ellipsoid, 0.8 mm long, sessile, centrally attached at the base; pericarp granulose, partially covered with minute papillae, hardly visible when dried; style mammiform when fresh, narrowly conical when dried. Fig. 17.

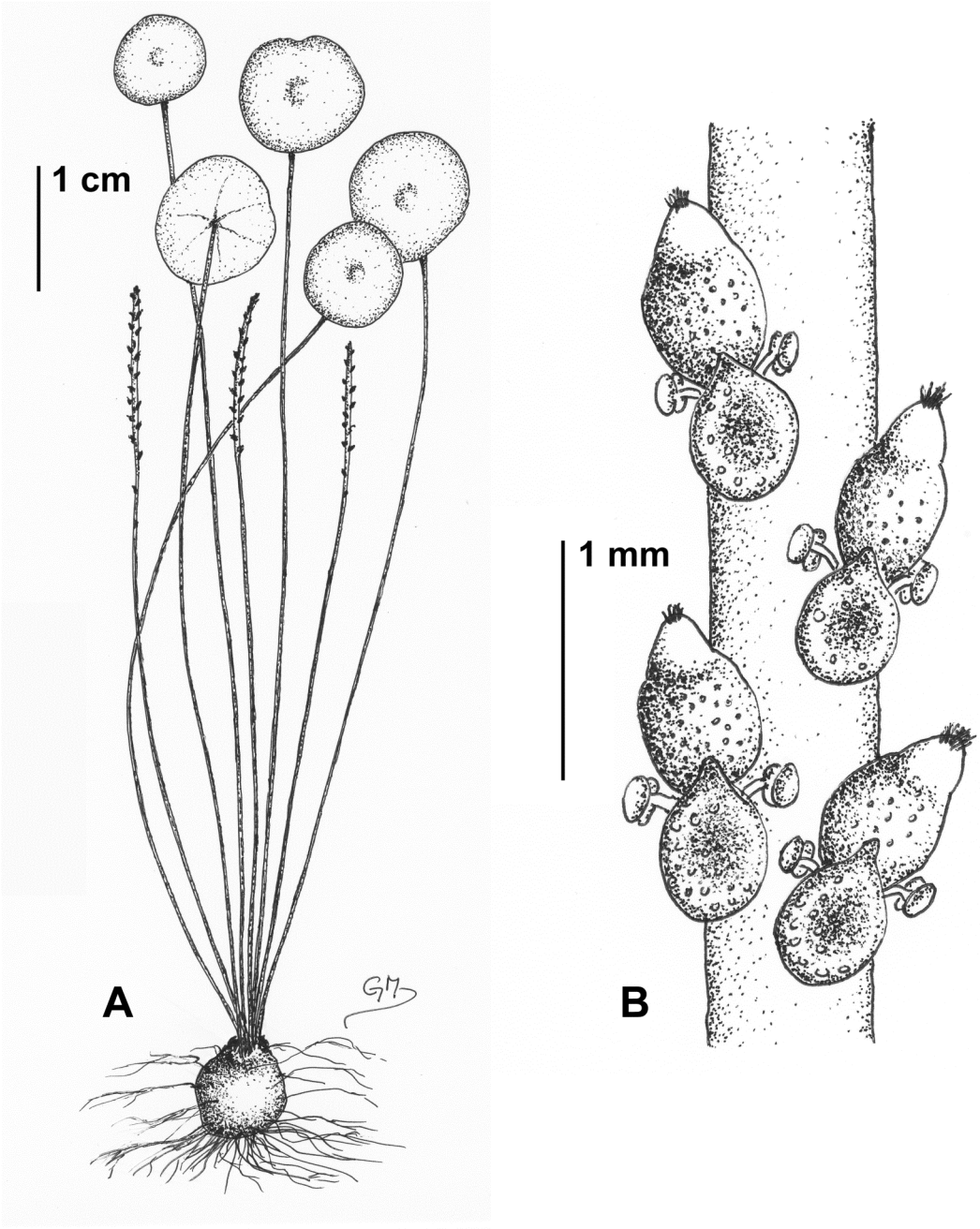


Figure 17. ***Peperomia umbrosa*: A, general habit; B, part of fruiting rachis.**

Other representative specimens examined -- **Peru**: Apurimac, prov. Andahuaylas, dist. Kishuara, along road Chincheros--Abancay, km 290.5, beyond Andahuaylas, 13°40'20.3"S, 73°11'48.4"W, 4,160 m, 27 Jan. 2009, *Samain et al.* 2009-067 (BR, GENT, USM); Junín, prov. Junín, along road La Oroya--Pasco, km 138, 11°17'27.9"S, 75°54'47.6"W, 4,190 m, 26 Feb. 2009, *Samain et al.* 2009-210a (USM).

Etymology -- The specific epithet refers to the shady habitat where the type collection was made.

Relationships -- This species is distinct in its slender general habit, its small, dark brown, tubers with rough surface and the long petioles of the small obscurely nerved leaves. Also *P. cerrateae* is characterized by small tubers but these have a yellowish and smooth surface and root at the apex. This species resembles *P. querococha*na to some extent. However, the leaves of the latter species have a distinct nervation and a tuber rooting only at the base. Phylogenetically *P. umbrosa* appears to be related to *P. polycephala* Trel., *P. peruviana* Dahlst. and *P. purpureonervosa* G.Mathieu (Fig. 1). *P. andina* Pino and *P. jalcaensis* Pino, two other geophytic *Peperomia* species, are growing abundantly on exposed spots near the *P. umbrosa* type locality.

Phenology -- The plant has been found above-ground in February.

Distribution and habitat -- The localities of the mentioned collections (departments of. Cajamarca, Apurimac and Junín) suggest a rather wide Peruvian distribution. The type collection was made from a population growing in a cave-like rock formation, dark and wet, in loamy soil together with liverworts. Additional collections mentioned herewith were found in crevices and at the base of rocks, in shady conditions but not as extreme as the type collection.

17. *Peperomia wernerrauhii* Pino & Samain, **sp. nov.**

Planta geophila a *P. andina* Pino differt tubere minore supra non caulescente, laminis foliorum minoribus subtus non purpurascentibus. – Type: Peru, Huánuco, prov. Huánuco, dist. Santa María del Valle, Cochapampa, near Marambuco, on rocks of steep canyon aside fields of Gregorio Espíritu, growing together with *P. naviculifolia* Trel. and *Echeveria andicola* Pino, 09º53'37"S, 76º09'03"W, 2,470 m, 27 Feb. 2009, *Samain et al.* 2009-214 (holo-: USM; iso-: BR, GENT).

Small herb, 5--14 (--20) cm tall. Tuber usually buried or partially exposed, globose, depressed, light grayish brown, 7-11 mm high, (0.7--) 1.1--1.5 (--2.2) cm diam, rooting at base, apex flat forming a darker central ring 3--7 mm diam. Leaves 6-9 (--12) per plant; petiole terete, light green with reddish longitudinal lines, 2.5--5(--9) cm x 0.9--1.2 mm diam, erect to slightly curved; lamina orbicular to widely ovoid, chartaceous, peltate a little below middle, (0.8--)1.2--2.2 cm diam, margin entire, apex minutely emarginate, adaxially concave or flat, centrally umbilicate, glossy green, obscurely 7-palmatinerved, nerves lighter in colour and not reaching margin, only central nerve from insertion of petiole to apex conspicuous, darker and sometimes there induplicate, abaxially greenish white. Spadices 8--14(--20) per plant, initially erect, then slightly curved; peduncle 4--8 cm x 0.8--

0.9 mm, light green or reddish; rachis 4--10(--14) cm x 1--1.6 mm, light yellowish green. Floral bracts peltate, elliptic, green, 0.8--0.9 mm long, 0.6--0.7 mm wide. Stamens with filaments 0.15 x 0.1 mm, terete, transparent; anthers ellipsoid 0.35--0.4 x 0.25--0.3 mm, red when young, dark brown when drying. Ovary ovoid, bright green, 0.6--0.7 x 0.5--0.6 mm; stigma globose, yellowish green 0.3 x 0.35 mm. Fruit ovoid, body 0.8--0.9 x 0.7--0.8 mm, brown; style widely conical, green, 0.3--0.4 x 0.35--0.45 mm; stigma dark. Fig. 18.

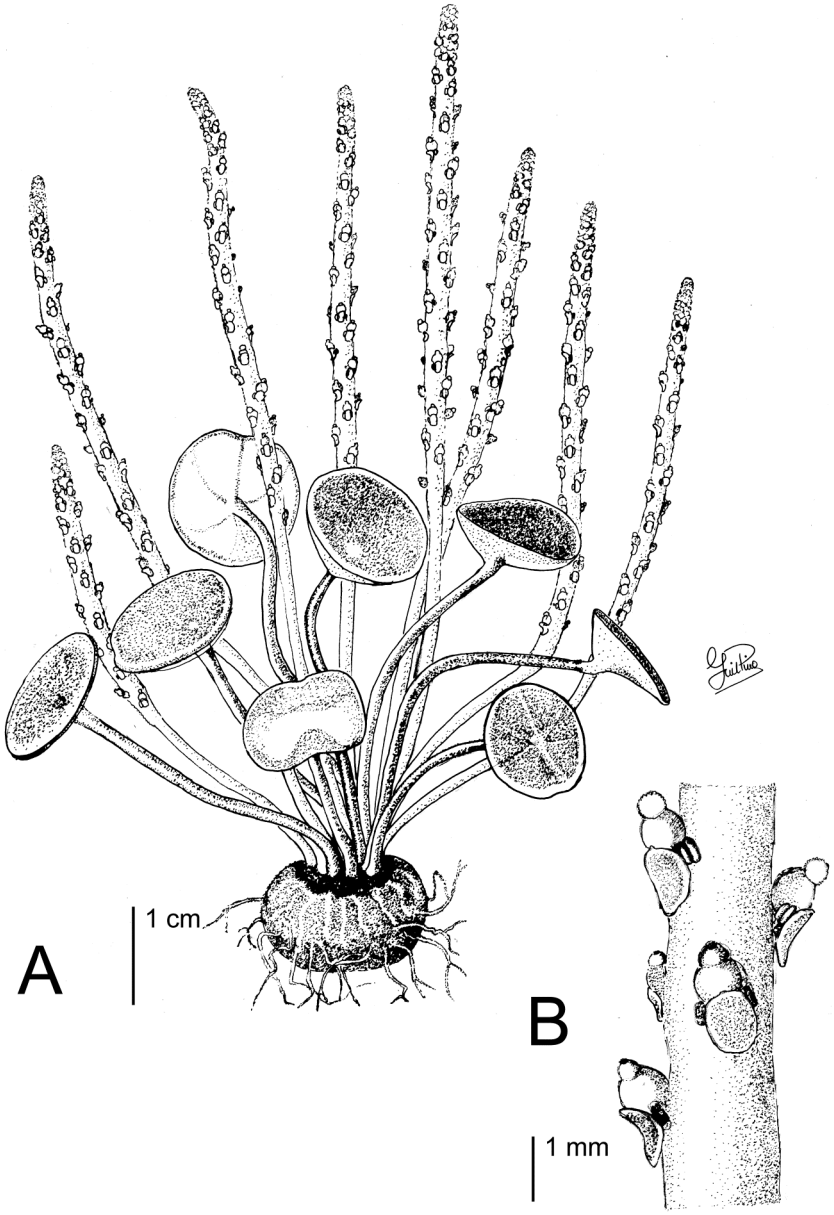


Figure 18. ***Peperomia wernerrauhii*: A, general habit; B, part of flowering rachis**.

Other representative specimens examined -- **Peru**: Huánuco, prov. Huánuco, dist. Chinchao, along road Pachachupan--Pillao, 4.5 km from the crossing with the road Huánuco--Chinchao km 34, 09º47'59"S, 76º03'27"W, 2,380 m, 1 Mar. 2009*, Samain et al.* 2009-248 (BR, GENT, USM); dist. Churubamba, Chinchobamba, 10º04'S 76º11'W, 2,200 m, 9 Apr. 2005, *Pino & Novoa* 1623 (Photos); prov. Huamalíes, dist. Chavín de Pariarca, Chavín, 2,800 m, *Rauh* 53869a (HEID).

Etymology -- The name stands for Werner Rauh (1914--2000), the German botanist who discovered this plant and described several *Peperomia* species from Peru and Ecuador. The name *P. wernerrauhii* differs from *P. rauhii* Hutchison, a herbarium name of *P. congesta* Kunth (Mathieu 2007: 419).

Relationships -- This species was discovered in 1983 by Werner Rauh in Chavín de Pariarca and was cultivated in Heidelberg Botanical Garden where he primarily identified it as *P. peruviana* and with that name he published a photo of this plant in 1989 (Rauh 1989). Since then it was not collected until we found it in several places of the department of Huánuco immediately pointing out its relationship with *P. andina*. Both species form identical depressed globose or discoid tubers with a black ring on the top where petioles and spadices emerge, although *P. andina* has larger tubers and eventually this ring can grow as a short conical stem in very old plants. The leaves of this new species are smaller, less markedly ovate, slightly concave instead of infundibuliform and they lack the purple hue of the abaxial side of *P. andina*. *P. wernerrauhii* is closely related to several other Andean species, amongst others *P. andina* (Fig. 1).

Phenology -- Leaves are present from November to April, spadices appear from January to March, fruits ripen from February to March.

Distribution and habitat -- It grows on rocks partially hidden by mosses or exposed, in the Department of Huánuco at 2200-2800 m.

Acknowledgement

We thank the authorities of Bolivia (permit number MDRAyMA-VBRFMA-DGBAP-UAVPS N° 046/08) and Peru (permit number 009-2009-AG-DGFFS-DGEFFS) for permission to collect material; Joaquina Albán, Carlos Bambarén, Roberto Bolaños, Brian Bates, Stephan Beck, Asunción Cano, Carmen Deza, Rodolfo Díaz, Gregorio Espíritu, Luisa Infante, Roobert Jiménez, Nilson Julcarima, Guido P. Lombardi, Masoliver Matos, Rosaisela Meneses, Betty Millán, Juan F. Montoya, Victor Morales, Gloria Rebaza, Leyda Rimarachin, Merli Saavedra, Isidoro Sánchez-Vega, Susanne Sivila and Anselmo Turpo for assistance with field work and logistics; Gerrit Davidse, Kanchi Gandhi and John Wiersema for their input on nomenclatural matters; Eric Tepe and Elmar Robbrecht for constructive comments; the Botanical Gardens of Ghent University and University of Technology Dresden for general support; Pieter Asselman for lab work; Karel Otten for databasing; Robert Maijer for provision of samples; and the curators of the herbaria at AAU, B, BH, BIGU, BM, BOLV, BR, C, CIB, CIIDIR, CM, CORU, CPUN, E, EBT, F, G, GB, GENT, GH, GOET, HEID, HUA, HUT, IBUG, IEB, ILL, INB, ISC, JE, K, L, LPB, LY, M, MA, MEXU, MICH, MO, NY, P, PH, PR, PRC, QCNE, S, SEL, TCD, U, UC, UPS, US, USM, VT, W, XAL and ZSS for the loan or permission to study material. This work was supported by the Research Foundation Flanders (FWO-Vlaanderen; FWO G.0172.07; FWO travel grants to the first author; the German Science Foundation (DFG NE681/5-1, DFG NE681/5-2); the Department of Biology, Ghent University; the Friends of the Botanical Garden Ghent and the Gesellschaft der Freunde und Förderer der TU Dresden, and the German Academic Exchange Service (DAAD PPP Colombia).

CHAPTER 2

The North American centre of *Peperomia* subgenus *Tildenia* diversity

This chapter is a modified version of:

Mathieu, G., Symmank, L., Callejas, R., Wanke, S., Neinhuis, C., Goetghebeur, P. & Samain, M-S.

New geophytic *Peperomia* (Piperaceae) species from Mexico, Belize and Costa Rica.

Revista Mexicana de Biodiversidad, 82, 357-382 (2011).

Summary

Peperomia subgenus *Tildenia* is a poorly known group of geophytic species occuring in seasonal habitats in two biodiversity hot spots (Mexico-Guatemala and Peru-Bolivia) with few species reported from the countries in between. Recent fieldwork combined with detailed study of herbarium specimens of this subgenus in Mexico and Central America resulted in the discovery of 12 new species, which are here described and illustrated. In addition, one formerly published variety is raised to species rank. Distribution, habitat and phenology data and detailed comparisons with other species are included, as well as an identification key for all species belonging to this subgenus in the studied area.

Introduction

The genus *Peperomia* Ruiz et Pav. includes approximately 1,600 species (Samain et al., 2009), and is one of the 10 most species-rich genera of angiosperms (Frodin, 2004). The largest diversity of the genus is encountered in America where it occurs in a wide range of habitats from southern USA to northern Argentina and Chile. Although it is generally thought that the majority of *Peperomia* species are epiphytic, an important but currently not yet adequately estimated number are terrestrial. Perhaps as a consequence of this generally accepted idea that most *Peperomia* species are epiphytes, the terrestrial species are undercollected and have been studied less intensively.
Peperomia subgenus *Tildenia* (Miq.) Miq. ex Dahlst. is a poorly known group of geophytic species. Our knowledge of these species is even more restricted than other terrestrial species of *Peperomia* as the geophytes are only above-ground during a few months of the year, usually the local rainy season. This reduces the chance of being collected unless one is specifically looking for them. The group is easily recognizable because of the presence of a tuber with petioles and peduncles originating directly from it (Wanke et al., 2006; Samain et al., 2009). Most species occur in seasonal habitats in two biodiversity hot spots (Peru-Bolivia and Mexico-Guatemala) with few species reported from the countries in between. The last detailed study dealing exclusively with subgenus *Tildenia* dates back more than a century (Hill, 1907). A comprehensive reinvestigation of this group has largely been hindered by fragmentary and insufficiently documented herbarium material. Many specimens lack tubers and/or inflorescences which are essential for proper identification. Furthermore, key information (e.g. color, texture) often got lost during the drying and storage process and habitat data are usually missing. Finally, the similar habit of most species of subgenus *Tildenia* hinders their identification. Observation of living plants in their habitat combined with meticulous reinvestigation of existing herbarium material a prerequisite for correct identification.

During this study it became obvious that subgenus *Tildenia* in this area includes many more species than initially assumed. In this study 12 species are newly described and one former variety is raised to species rank. Collections of these newly described species can be ranked in three categories:
1) collections which already had been recognized as belonging to new species by other botanists but which remained undescribed,
2) collections representing these new species that had been incorrectly identified, often as a widespread species, despite the fact that the specimen did not at all fit to the description of this common species,
3) collections we made at localities where to our knowledge nobody had ever collected a tuberous species of *Peperomia* before, proving that this group is generally overlooked. A key to all species of *Peperomia* subgenus *Tildenia* we currently recognize in Mexico and Central America is presented in expectation of a future revision of the entire subgenus *Tildenia*.

Material and Methods

Field work, dedicated nearly exclusively to this group, was recently carried out in close collaboration with local botanists in Mexico and Costa Rica. This study is based on the analyses of about 100 of our own recent collections and approximately 600 additional specimens from 57 herbaria. For our own collections, specimens of different developmental stages as well as longitudinal sections of the tubers were preserved. When possible, infructescences with mature fruits were stored in 70% alcohol. After careful cleaning and digital imaging of all aerial and subterranean parts, plants were immediately pressed and dried using a hot air drier that kept the temperature of the specimens below 50°C.

Results and Discussion

Species concept

Tildenia was originally described by Miquel (1843a) as a genus – then consisting of one species, *Tildenia mexicana* (= *Peperomia mexicana*) nom. cons. prop. (Mathieu, 2009: 1374) – within the family Piperaceae. Shortly afterwards, Miquel (1843b) also transferred *Peperomia umbilicata* Ruiz et Pav. to the new genus but named it *Tildenia peruviana* Miq. In his Systema Piperacearum, he recognized *Tildenia* as a subgenus ['sectio'] of genus *Peperomia* within the tribe Peperomieae (Miquel, 1843c-1844). Dahlstedt (1900) recognized a much wider circumscribed subgenus *Tildenia* and merged all *Peperomia* species with peltate leaves without noting the occurrence of tubers (Samain et al., 2007). Hill (1907) finally recognized the tuberous geophytic species within the subsection *Geophila* of the subgenus *Tildenia* sensu Dahlstedt (Samain et al., 2007). However, recent phylogenetic analyses of the genus *Peperomia* based on coding and noncoding plastid DNA show that subsection *Geophila* is clearly separated from the remaining species of *Tildenia* sensu Dahlstedt (1900), forming a distinct monophyletic clade (Wanke et al., 2006, 2007; Samain et al., 2009). The correct name to be used for this clade is *Peperomia* subgenus *Tildenia* (Miq.) Miq.Hill (1907) distinguished 22 different geophytic species from Mexico and adjacent countries as well as from South America, of which 17 are still accepted today. *Peperomia cotyledon* needs to be excluded from this group based on molecular data (Wanke et al., 2006; Samain et al., 2009), which confirmed earlier morphological evidence (Dahlstedt, 1900).

Since Hill's revision (1907), more than 15 species belonging to this group were described (Trelease, 1922, 1936; Rauh and Kimnach ,1987; Pino, 2004, 2008; Pino et al., 2005; Véliz, 2007). Several species of Trelease (1922) from Mexico and Costa Rica were published with scanty descriptions based on herbarium specimens and have appropriately been synonymized with more widespread species (Burger, 1971; Nyffeler and Rowley, 2002). Similar to the species described in this paper, the more recently described species were all based on fresh field collections, generally by the author himself, and clearly are segregate species. This is not only supported by distinct morphological characters but also by our ongoing molecular investigations (unpublished), which in addition confirm the relatively narrow species concept applied throughout *Peperomia*.

Finally, it is also worth noting that most of the newly described species are narrow endemics (Fig. 1), which might be explained as 1) a result of undercollection due to the inconspicuousness of the plants or difficult accessibility and seclusion of some of their localities, or 2) a genuinely restricted distribution pattern, perhaps related to the scarcity of suitable habitats. Further field study is required to elucidate this interesting pattern which is not only applicable in *Peperomia* subgenus *Tildenia* but in many terrestrial *Peperomia* species.

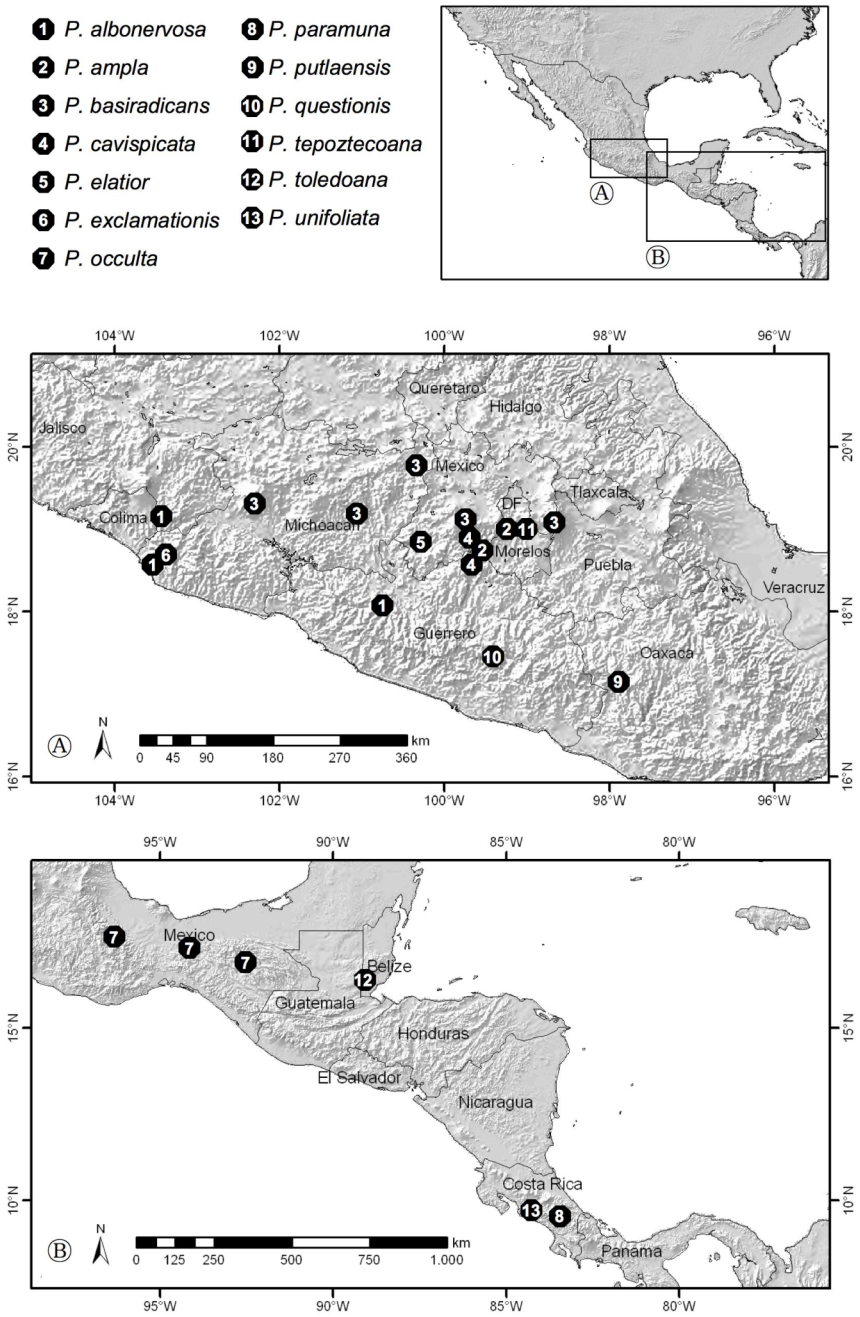


Figure 1. Distribution map of the species here described.

Key to the species of *Peperomia* subgenus *Tildenia* (Miq.) Miq. in Mexico and Central America

1. Inflorescence compound. 2
  2. Leaves with black pellucid dots. *P. toledoana* Callejas
  2. Leaves without black pellucid dots. *P. claytonioides* Kunth
1. Inflorescence simple (but sometimes fasciated or split near the apex). 3
  3. Tuber with several large contorted trunk-like outgrowths. *P. tepoztecoana* G. Mathieu
  3. Tuber without trunk-like outgrowths. 4
    4. Roots at base of tuber. 5
      5. Leaves centrally peltate. *P. basiradicans* G. Mathieu
      5. Leaves peltate near the base of the lamina. *P. cuchumatanica* Véliz
    4. Roots at apex of tuber. 6
      6. Plant with 1(-2) leaves and one inflorescence. 7
        7. Fruit pedicellate, style 0,3 mm long. *P. unifoliata* Callejas
        7. Fruit sessile, style 2 mm long. *P. linaresii* Véliz
      6. Plant with three or more leaves and/or two or more inflorescences. 8
        8. Tuber irregularly shaped and rhizomatous. 9
          9. Leaves not or slightly peltate, fruit short pedicellate. *P. mexicana* (Miq.) Miq.
          9. Leaves peltate, fruit sessile. 10
            10. Leaves peltate at about ¼ of the leaf length from the base. *P. macrandra* C.DC.
            10. Leaves centrally peltate or rarely a little below the center. 11
              11. Style present, ovary smaller than the prominent style. *P. ampla* (Trel.) G. Mathieu
              11. Style absent. 12
                12. Fresh rhizome whitish with abundant fibrous roots. *P. moralesii* Véliz
                12. Fresh rhizome brownish without fibrous roots. *P. monticola* Miq.
        8. Tuber more or less globose, not rhizomatous. 13
          13. Leaves not peltate or peltate close to the base of the lamina. 14
            14. Leaves subpeltate, style 1.0-1.5 mm long. *P. mexicana* (Miq.) Miq.
            14. Leaves peltate, style 0.1-0.2 mm long. *P. putlaensis* G. Mathieu
          13. Leaves clearly peltate. 15
            15. Leaves deltoid. 16
              16. Rachis generally straight, the distal end sometimes curved; fruit pedicellate, fruit wall
                not papillate or minutely papillate. *P. exclamationis* G. Mathieu
              16. Rachis curved nearly in its entirety; fruit sessile, fruit wall distinctly papillate. *P. questionis* G. Mathieu
            15. Leaves (irregularly) orbicular to (widely) ovate. 17
              17. Rachis fusiform. 18

18. Rachis much longer than peduncle, fresh plant with conspicuous cilantro smell.

P. cavispicata G. Mathieu

18. Rachis much shorter than peduncle, fresh plant without conspicuous cilantro smell.

P. elatior G. Mathieu

17. Rachis filiform to cilindriform, tapering at its apex only. 19

19. Leaves thin, translucent. 20

20. Leaves peltate at ca 1/3 of the leaf length from the base, fruits narrowly ellipsoid, pedicellate. *P. occulta* G. Mathieu

20. Leaves centrally peltate, fruits (sub-)globose, sessile.

P. gracillima S. Watson

19. Leaves membranous-chartaceous-succulent, not translucent. 21

21. Leaf nerves differently colored than lamina. 22

22. Leaf nerves and small zone along them adaxially white.

P. albonervosa G. Mathieu

22. Leaf nerves and small zone along them abaxially purple.

P. ovatopeltata C.DC.

21. Lamina entirely green, the leaf nerves sometimes lighter green. 23

23. Fruit long pedicellate. *P. pedicellata* Dahlst.

23. Fruit sessile 24

24. Floral bract ovate-lanceolate, the apex acuminate.

P. schizandra Trel.

24. Floral bract orbicular-elliptic, apex sometimes short acuminate 25

25. Rachis extremely short compared to peduncle.

P. paramuna Callejas

25. Rachis and peduncle of more or less equal length. 26

26. Fruit cylindrical *P. monticola* Miq.

26. Fruit ellipsoid-globose. 27

27. Fruit ellipsoid, style conical *P. bracteata* A.W. Hill

27. Fruit globose, style flattened *P. painteri* Trel.

Taxonomic treatment

1. *Peperomia albonervosa* G.Mathieu, n. sp. (Figs. 2, 3)

Synonyms: *Peperomia bajucosensis* Trel., nom herb. (Mathieu 2007: 45), *Peperomia placeresana* Trel., nom. herb. (Mathieu 2007: 382)

Type: MEXICO. Michoacán: mun. Aquila, just beyond Puente Aquila, along the road to the Pacific Coast, 18°35'05.3"N, 103°30'55.4"W, 180 m, 25 Jul. 2007, *M-S. Samain et al. 2007-046* (holotype: BR!; isotypes: G!, GENT!, K!, MEXU!, MO!).

A Peperomia schizandra *Trel. differt laminis atrovirentibus albonervosis supra reticulatis.*

Small herb, ca. 5 cm tall in vegetative stage, entirely glabrous. Tuber globose, slightly flattened, to 8 mm diameter, the surface smooth, yellow, rooting at apex. Leaves 1-5(-20) per plant; petiole (3-)5-7(-12) cm long; lamina membranous, irregularly orbicular, to 7.5 cm diameter, peltate centrally or a little below center, the apex rounded or discretely obtuse, rarely acute, 8-palmatinerved with 3 nerves running into apical half of lamina, 2 nerves running almost laterally and 3 nerves running into basal half, the main nerves distally branching, the nerves and a small zone along nerves white or creamy adaxially, distinctly contrasting with deep green color of remainder of the lamina, green or purple between nerves abaxially, adaxially reticulate. Spadices 1-5 per plant; peduncles 5-7(-10) cm x 1.5 mm; rachis 2-5(-10) cm x 2 mm, usually distinctly shorter than peduncle, tapering at apex, white or whitish green, the distal 1 cm purple, or entire rachis pink-purplish, loosely flowered. Floral bracts elliptic, 0.8 x 0.6 mm, centrally peltate, the apex acuminate, the margin slightly undulate. Stamens with filaments 0.4 mm long when fresh; anthers 0.45 x 0.35 mm, pink purplish before releasing pollen, later shriveled and brown. Ovary ellipsoid, initially smaller than style and entirely covered by floral bract; stigma terminal, subglobose fimbriate. Fruits ellipsoid, the body 1-1.5 x 0.7-1 mm, centrally attached at base, on short and broad pseudopedicel when mature; pericarp verruculose when fresh, reticulate when dried, dark brown; style mammiform and minutely verruculose when fresh, conical and rather granulose when dried, 0.2-0.3 mm long, dark brown.

Taxonomic summary

Additional specimens examined. MEXICO. Guerrero: distr. Mina, Placeres, under trees, 350 m, 24 Jul 1936, *G. B. Hinton 9145* (GH!, ILL!, K!, MO!, NY!, US!). Jalisco: 13 miles N of Santiago (Colima), Río Cihuatlán, 175-200 m. 31 Jul. 1957, *R. McVaugh 15939* (MICH!); mun. Pihuamo, along highway 110, E of Huizache, 19°10'23.3"N, 103°28'50.9"W, 660 m, 21 Jul 2007, *M-S. Samain et al. 2007-039* (BR! GENT!, MEXU!). México: mun. Temascaltepec, Bejucos, 610 m, 4 Jul. 1933, *G. B. Hinton 4279* (ILL!, K!, NY!).

Etymology. The specific epithet refers to the white to creamy adaxial nervation pattern of the leaves, a distinct feature of this species.

Distribution and habitat. *Peperomia albonervosa* is reported from tropical deciduous forest (bosque tropical caducifolio and subcaducifolio *sensu* Rzedowski, 2006) in Jalisco, Guerrero and México. It occurs at lower elevations (175-660 m) in shallow soil in crevices in rock formations. *Peperomia schizandra* grows in a similar habitat and vegetation type, but at slightly higher average elevation (the highest locality is at 1100 m) and with a broader distribution as it also occurs in Colima, Michoacán and Oaxaca, and northwards up to Nayarit.

Phenology. The plant has been found above-ground in July, flowering and fruiting.

Remarks

This species is morphologically similar to *P. schizandra* Trel. from which it differs by the dark green, white nerved and adaxially reticulate lamina. Although these characters are obvious in living plants (Fig. 3), they tend to fade in herbarium specimens, and many collections are often misidentified as *P. schizandra*.

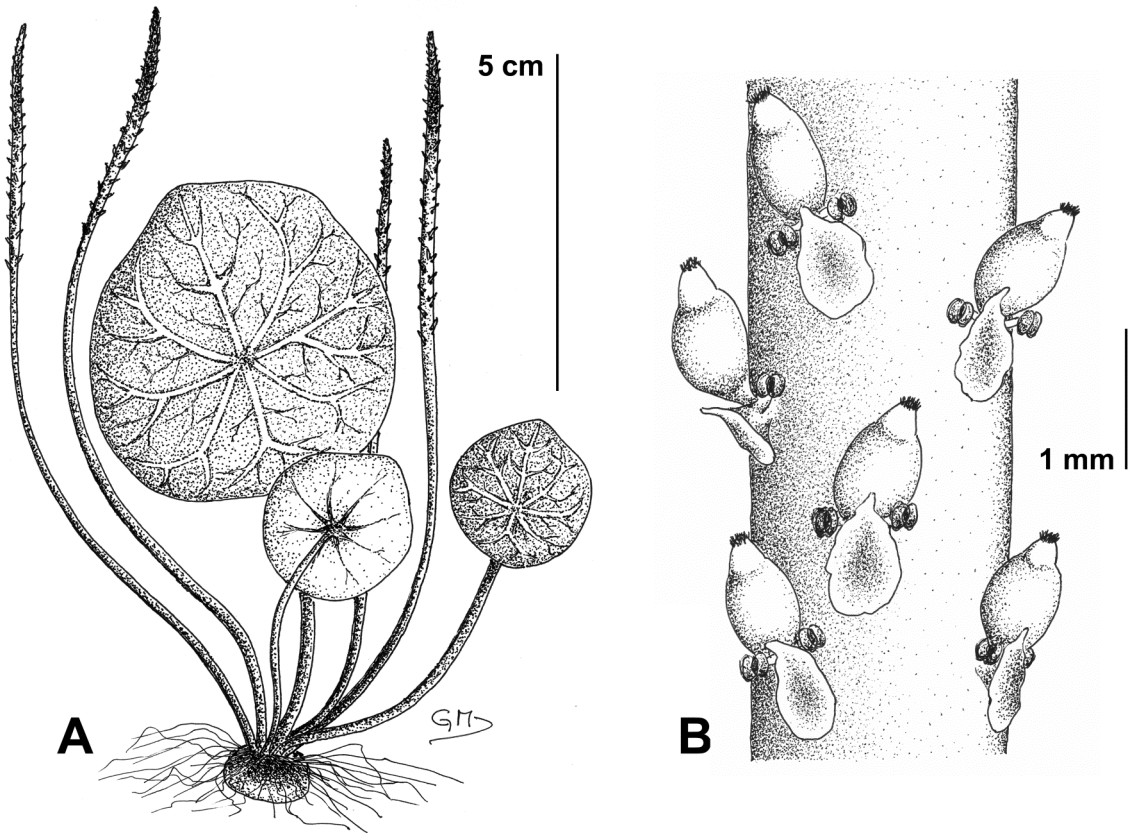


Figure 2. ***Peperomia albonervosa*. A, general habit. B, part of rachis.**

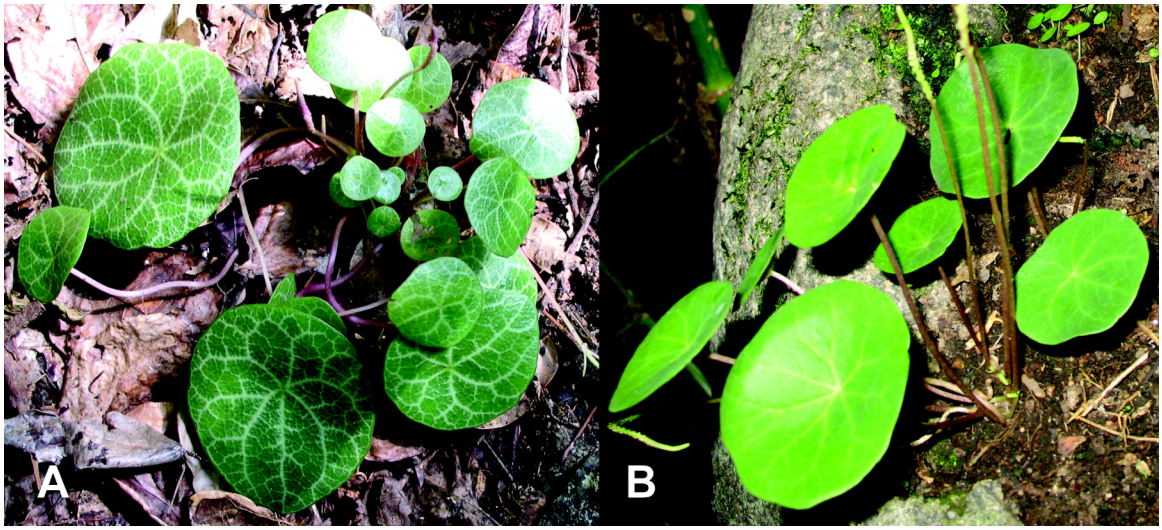


Figure 3. **A. *Peperomia albonervosa* in situ, B. *Peperomia schizandra* in situ.**

2. *Peperomia ampla* (Trel.) G.Mathieu, n. stat. (Fig. 4)

Basionym: *Peperomia macrandra* C.DC. var. *ampla* Trel., Botanical Gazette 73: 140. 1922.

Type: MEXICO. Morelos: canyon above Cuernavaca, 6500 ft [1980 m], 21 Jul 1904, *C. Pringle 13282* (holotype: US!; isotypes: BH!, F!, GH [2]!, MICH [2]!, US [2]!).

Robust herb, up to 25 cm tall in vegetative stage, entirely glabrous. Tuber irregular, roots at the apex rhizomatous, up to 5 cm or more diam., sometimes lobed and with leaves and inflorescences originating from 1-3 growing points. Leaves 2-5 per plant; petiole up to 25 x 0.5 cm; lamina chartaceous, orbicular or slightly ovate, up to 10 cm diam., centrally peltate or rarely a little below center, the apex acute to acuminate, 8-(9)-10-palmatinerved, main nerves branching, reticulately nerved in between, a peripheral anastomosing nerve running along entire margin. Spadices 1-4 per plant; peduncle up to 8 x 0.5 cm, distinctly shorter than the petioles; rachis up to 40 x 0.6 cm, loosely flowered. Floral bracts orbicular to elliptic, 0.9 x 0.6 mm, centrally peltate, the apex acuminate, the margin slightly undulate, minutely hyaline, glandular-dotted. Stamens with filaments short and thick when young, longer and slender when maturing, finally wilted but still present, 0.3-0.9 mm long; anthers 0.2 x 0.3 mm. Ovary smaller than its prominent, ovoid, glandular-dotted style, resulting in a typically mushroom-shaped pistil; stigma fimbriate, terminal on a small, slightly abaxially inclined, stigmatal plateau. Fruits ellipsoid, body 0.8-0.9 x 0.7 mm, sessile, centrally attached at base; pericarp granulose, basally papillate; style conical, 0.3-0.4 mm long.

Taxonomic summary

Additional specimens examined. MEXICO. México: 'Barranca de Tonatico', 1420 m, 26 Sep. 1954, *E. Matuda et al. 31682* (MEXU!). Morelos: mun. Cuernavaca, Sierra de Morelos, mixed forest in canyon, 1975 m, 2 Aug. 1969, *G. Hinton et al. 17229* (MICH!); Valle del Tepeite, Huitzilac, Aug. 1930, *E. Lyonnet 667a* (ILL!, MEXU [2]!, US!), Valle del Tepeite, Sierra de Ocuila, Sep. 1930, *E. Lyonnet 667b* (NY!); Valle del Tepeite, Huitzilac, Jul. 1933, *E. Lyonnet 667c* (MEXU [2]!); Santa María, 10 Aug. 1933, *E. Lyonnet 667d* (MEXU [2]!); Valle del Tepeite, Sep. 1933, *E. Lyonnet 667e* (GH!, K!, MEXU [2]!, MO!) [based on the several collection dates and localities, *Lyonnet 667* includes five different gatherings, which have been annotated by us chronologically a-e].

Distribution and habitat. *Peperomia ampla* is known from tropical deciduous forest and pine-oak forest in the states of México and Morelos, at approximately 1400-2000 m elevation.

Phenology. The plant has been found above-ground from July to September, flowering and fruiting.

Remarks

Trelease (1922: 140) mentioned the diagnostic characters of variety *ampla* by one sentence: "A large form with round-ovate leaves as much as 8 x 10 cm and shorter beak on the fruit". He apparently had only seen the US specimen, which he designated as the holotype. Close inspection of the isotypes reveals that there is more morphological difference between the variety and the species than between some other geophytic taxa. This is also confirmed by the other collections mentioned above and therefore, variety *ampla* is raised here to species rank and an emended description is provided.

From *P. ampla*, *P. macrandra* C.DC. differs in its more slender general habit, in its leaves (ovate, 4-6 x 2.5-4 cm, peltate at about ¼ of the leaf length from the base, apex usually longer acuminate), spadices (peduncle distinctly longer than rachis) and fruits (ellipsoid-cylindrical, pedicellate, pericarp not papillate, style cylindrical). Both are obviously not identical as has been suggested by Nyffeler and Rowley (2002: 366). In its vegetative stage *P. ampla* rather resembles *P. cavispicata* G.Mathieu. Nevertheless, the latter is distinct in its fusiform rachis and more slender petiole. The robust rachis in *P. ampla* is longer, has about the same diameter over its entire length and is supported by a thick peduncle. Although both species exhibit pistils with a prominent style, their shape is broadly ellipsoid to ovoid in *P. ampla* (Fig. 4) whereas it is narrowly ellipsoid to cylindrical in *P. cavispicata*. *Peperomia ampla* has a large, irregular, rhizomatous tuber whereas it is rather small and globose in *P. cavispicata*.

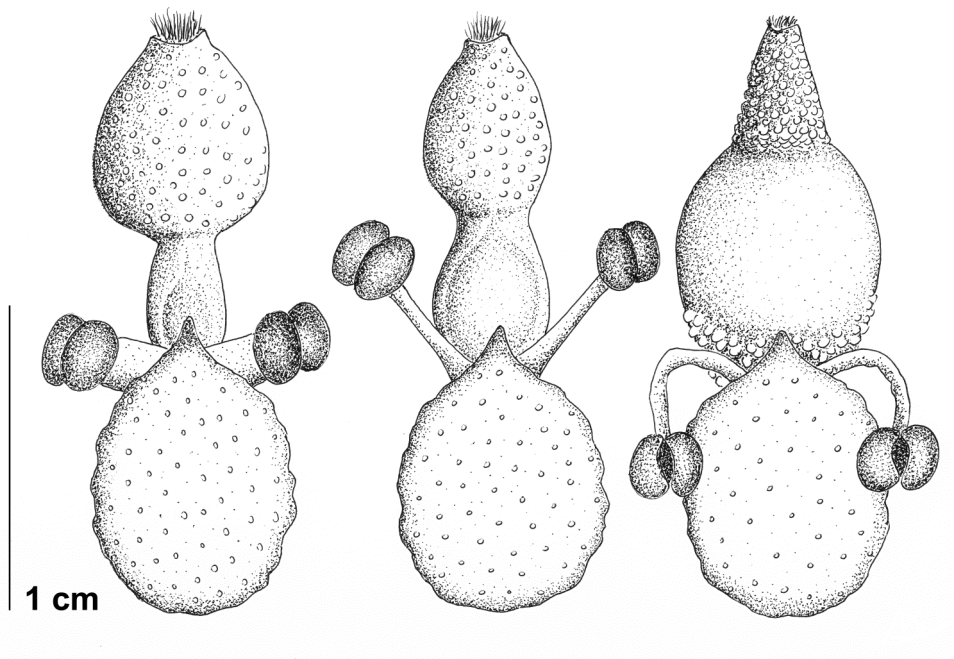


Figure 4. ***Peperomia ampla*, different stages of maturing fruit (less mature to the left, more mature to the right).**

3. *Peperomia basiradicans* G.Mathieu, n. sp. (Fig. 5)

Type: MEXICO. México: Nevado de Toluca, N slope of the volcano, just E of where the road crosses the timberline, 19°07'29.2"N, 99°45'38.6"W, 3,920 m, 18 Jul. 2007, *M-S. Samain et al. 2007-036* (holotype: BR!; isotypes: GENT!, MEXU!).

A Peperomia gracillima *A. W. Hill differt tubere, e base radicanti, parte basali laevi flavaque, parte apicali aspera brunneaque, bracteis floralibus antheribusque majoribus, filamentis bis brevioribus.*

Delicate herb, 2-5 cm tall in vegetative stage, entirely glabrous. Tuber globose when young, yellow, smooth, to 8 mm diam., older tuber same diameter but to 1.5 cm tall, sometimes apically lobed, the basal part yellow and smooth, the apical part rough and brown, rooting at base. Leaves 1-10 per plant; petiole to 3-4 cm in exposed locations, delicately elongated to 7 cm when growing in mosses; lamina orbicular, 0.6-1.6(-2) cm diam, centrally peltate, the apex rounded or obtuse with acute or acuminate tip. Spadices 1-15 per plant; peduncle (3-)4-5(-10) cm; rachis 2-7 cm, usually same length or longer and slightly thicker than the peduncle, loosely flowered. Floral bracts elliptic, 0.8-0.9 x 0.6 mm long, centrally peltate, the apex acute or acuminate. Stamens with filaments 0.3 mm long; anthers 0.2 mm long. Ovary ellipsoid; stigma terminal on small and slightly oblique stigmatal plateau. Fruits ellipsoid, 0.8-0.9 x 0.6 mm, sessile to subsessile on pseudopedicel less than 0.3 mm long; pericarp granulose; style short-conical, 0.2 mm long.

Taxonomic summary

Additional specimens examined. MEXICO. México: Ojos de Agua, Nevado de Toluca, 12,000 ft [3,660 m], 10 Jul. 1938, *E. Balls 4977* (E!, GH!, K!, US!); Nevado de Toluca, N side, 0.7 mi E of where road goes above timberline, 3,985 m, 28 Jul. 1958, *J. Beaman 1922* (GH!, NY!, US!); Amecameca, road Amecameca – Popocatepetl between km 76 and 77, on steep bank of gorge of small stream, locally fairly abundant, ca. 3,250 m, 2 Aug. 1958, *J. Beaman 2072* (MICH!, NY!); road Amecameca – Tlamacas between km 15 and 16, narrow canyon on slope of Popocatepetl volcano, 19°05'16.0"N, 98°40'45.2"W, 3,150 m, 11 Jul. 2007, *M-S. Samain et al. 2007-009* (BR!, GENT!, MEXU!). Michoacán: mun. Angangueo, Sierra Chincua, Reserva de la Biósfera Mariposa Monarca, 19°40'19"N, 100°17'30"W, 3,372 m, 31 Jul. 2001, *M. G. Cornejo & G. Ibarra 495* (IEB!, XAL!); Mt. Tancítaro, peak on E slope, in soil filled crevices in rocks under scattered tall pines, 3,100 m, 13 Aug. 1961, *D. De Jong & E. Longpre 1053* (F!); loc.cit., common on moist cliffs, open pine forest, 10,500 ft [3,200 m], 22 Jul 1941, *W. Leavenworth & H. Hoogstraal 1161* (F!, MO!).

Etymology. The specific epithet refers to the position of the roots at the base of the tuber, which is atypical among the Mexican geophytic *Peperomia* species, in which the roots generally originate at the apex of the tuber.

Distribution and habitat. Known collections are from the Trans-Mexican Volcanic Belt in the states of México and Michoacán where the species appears to occur on volcano slopes at 3,000-4,000 m in rather moist rocky habitats, near the upper limit of pine forest or above the timberline.

Phenology. The plant has been found above-ground in July and August, flowering and fruiting.

Remarks

Peperomia basiradicans resembles *P. gracillima* A. W. Hill, another delicate Mexican geophyte. The tuber of the latter is rooting at the apex and lacks a basal smooth yellow part and an apical rough brown part. It also differs in its floral characters (floral bracts smaller, filaments about twice as long as in *P. basiradicans*, anthers smaller). *Peperomia basiradicans* prefers higher elevations, whereas *P. gracillima* usually occurs between 1500-2500 m. The basal root position, common within the South American species, is quite rare in the Mexican and Central American species. Apart from *P. basiradicans*, it also occurs in *P. cuchumatanica* from Guatemala.

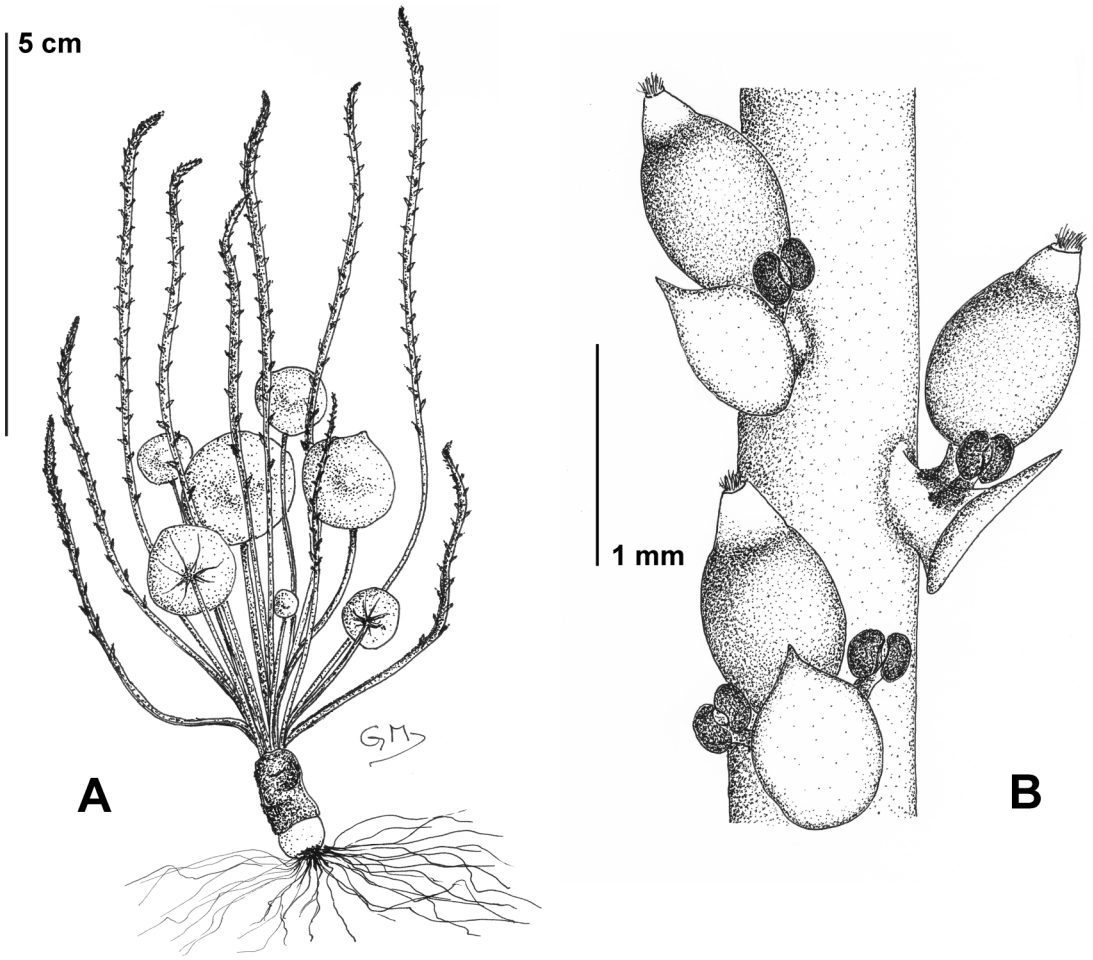


Figure 5. ***Peperomia basiradicans*. A, general habit. B, part of fruiting rachis.**

4. *Peperomia cavispicata* G.Mathieu, n. sp. (Fig. 6)

Type: MEXICO. Guerrero: mun. Taxco, Tenango del Paraiso, Chacoalco spring in village center, bend of road to La Cascada, 18°33'46.4"N, 99°37'17.4"W, 1,000 m, 15 Jul. 2007, *M-S. Samain et al. 2007-017* (holotype: BR!; isotypes: GENT!, MEXU[2]!).

A speciebus geophilis notis distincta rhachidi crassissima fusiformique, interna spongiosa cavitate centrali; a Peperomia elatior *G.Mathieu differt rachi longiore pedunculo brevioreque, stylo cylindrico, baccis sessilibus.*

Large herb, 10-25 cm tall in vegetative stage, entirely glabrous, all parts giving off a strong cilantro smell when crushed (Cilantro is the local name for *Eryngium foetidum* L. and/or *Coriandrum sativum* L., both commonly used in Latin American cooking and with a very distinctive smell). Tuber subglobose, to 1.3 cm diam and 1 cm tall, rooting at apex. Leaves 1-6(-10) per plant; petiole 5-13 long, sometimes reddish; lamina membranous, orbicular to ovate, to 12 cm diam., peltate a little below center, the apex usually distinctly acute, rarely rounded, 8(-10)-palmatinerved, with 3 nerves running into apical half of lamina, 2 nerves running almost laterally and 3(-5) nerves running into basal half, light green. Spadices 1-6(-9) per plant; peduncle 3-5 cm, sometimes reddish; rachis 5-12(-15) x 1.4 cm, long fusiform, sometimes about same length but usually 2-3 times as long as peduncle, pale green, filled with spongy tissue except for central cavity. Floral bracts orbicular to elliptic, ca. 0.9 mm long, peltate, the apex acuminate. Stamens with long filaments, initially 0.2-0.3 mm, then filiform, 0.7-1.0 mm, finely wilted but persistent; anthers 0.2-0.3 mm long. Ovary ellipsoid and smaller than its prominent, 0.7-0.8 mm long and slightly ellipsoid to cylindrical, hyaline glandular-dotted style; stigma terminal on small, slightly abaxially inclined stigmatal plateau. Fruits ellipsoid, ca. 0.9 mm long, sessile; pericarp granulose; style long-conical.

Taxonomic summary

Additional specimens examined. MEXICO**.** Guerrero: mun. Taxco, N of Taxco, N side of Chacoalco river, on cliffs, competing with *Sedum frutescens*, SE exposure, 1855 m, 25 Aug. 1955, *R. Clausen s.n.* (BH!, MEXU!, NY!); 10 miles NE of Taxco, 1770 m, 10 Jul. 1940, *C. Hitchcock & L. Stanford 7074* (US!); above and E of Taxco on trail to Chacualco, 5800-6200 ft, 15 Aug. 1948, *H.E. Moore & C.E. Wood 4607* (NY!); road Taxco – Ixcateopan, 5 km SW of Taxco, Lanada, humid canyon, on rocks, 1,790 m, 7 Jul. 1982, *E. Martínez & J. Soto 1243* (MEXU!); México: mun. Coatepec Harinas, near Villa Guerrero, along trail into Barranca de Texalotango from Rancho Santo Tobias, 21 Jul. 1945, *C. Gilly et al. 14* (MICH!); Villa Guerrero, Rancho Santo Tobias, small canyon reached by dirt road crossing corn fields, SE of the greenhouses of the ranch, 18°55'07.4"N, 99°37'38.6"W, 1,960 m, 16 Jul. 2007, *M-S. Samain et al. 2007-026* (BR!, GENT!, MEXU!).

Etymology. The specific epithet refers to the central cavity in the thickened rachis, a remarkable feature of this species.

Distribution and habitat. Known from the Mexican states Guerrero and México in tropical deciduous forest (bosque tropical caducifolio *sensu* Rzedowski, 2006) between 1,000-2,000 m elevation. It usually occurs in small canyons or moderately shaded locations where it grows on rock walls, often in little substrate.

Phenology. The plant has been found above-ground in July and August, flowering and fruiting.

Remarks

Peperomia cavispicata has to be distinguished from *P. elatior* G.Mathieu, which also has a thickened rachis. However, inflorescences of the latter species exhibit a long peduncle and a short rachis whereas in *P. cavispicata* it is exactly the opposite. The leaves of both species are similar although they are generally larger in *P. cavispicata*. The sessile fruit in *P. cavispicata* is easily distinguished from the pedicellate one in *P. elatior*. The pistil of *P. cavispicata* is distinct in the slender style whereas in *P. elatior* it is ellipsoid to obovoid and shows a particular large stigmatal plateau.

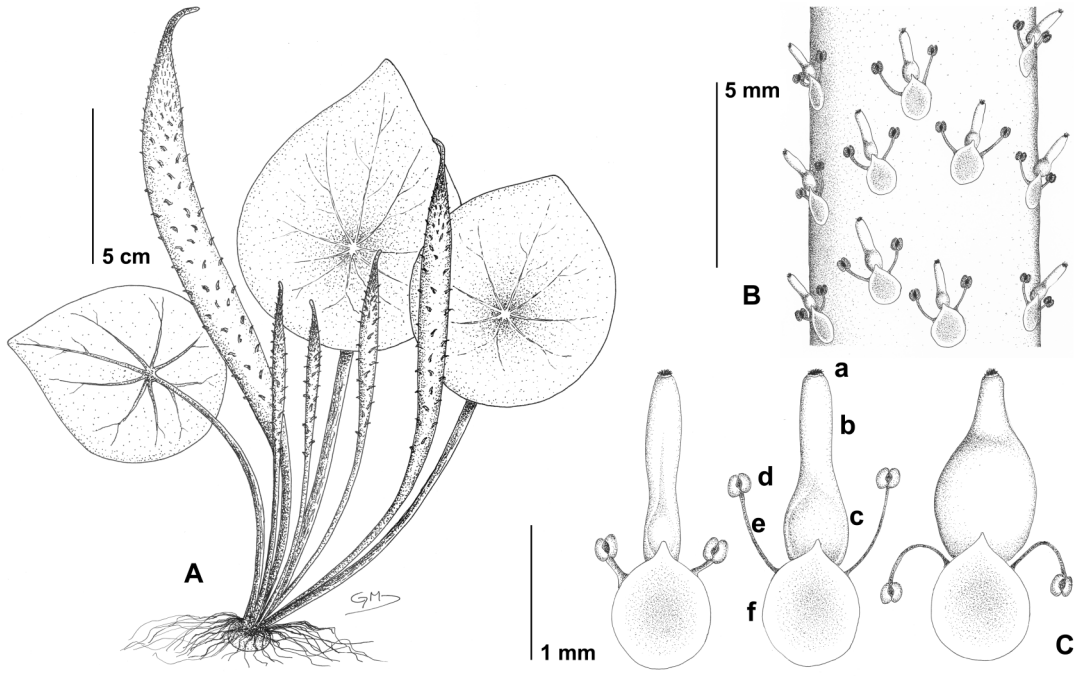


Figure 6. ***Peperomia cavispicata*. A, general habit. B, part of flowering rachis. C, different stages of developing fruit (young to the left, older to the right). a, stigma on small stigmatal plateau. b, style. c, ovary. d, anther. e, filament. f, floral bract.**

5. *Peperomia elatior* *G*.Mathieu, n. sp. (Fig. 7)

Type: MEXICO. México: distr. Temascaltepec, mun. Luvianos, road El Estanco – Nanchititla between km 6 and 7 (just beyond the village El Salitre), small population on vertical rock wall, 18°52'33.4"N, 100°17'09.0"W, 1,500 m, 17 Jul. 2007, *M-S. Samain et al. 2007-035* (holotype: BR!; isotypes: GENT!, MEXU!).

A speciebus geophilis notis distincta rhachidi crassissima fusiformique; a Peperomia cavispicata *G.Mathieu differt rachidi breviore petiolo longioreque et baccis pedicellatis majoribus stigmatiferisque.*

Large herb, up to 15 cm tall in vegetative stage, entirely glabrous. Tuber globose or slightly flattened, to 1 cm diam, rooting at the apex. Leaves 1-5 per plant; petiole 6-14 cm long, sometimes reddish; lamina membranous, drying translucent, ovate to orbicular, (3-)4-8(-9) cm long and a little less wide, length/width ratio 1.1 to 1.2, the apex acute to broadly acuminate, the base rounded, peltate a little below the center, 8-palmatinerved, distinct abaxially, with 3 nerves running into apical half of lamina, 2 nerves running almost laterally and 3 nerves running into basal half, the main nerves branching distally. Spadices 1-7 per plant; peduncle slender, 14-21 cm, sometimes reddish; rachis fusiform, (2-)3-6(-10) x 0.8 cm, filled with spongy tissue, a central cavity absent, loosely flowered. Floral bracts elliptic to almost orbicular, 0.8-1.0 x 0.7-0.9 mm, peltate, the apex acuminate or sharply acuminate in young bracts at spadix apex, the margin entire. Stamens deciduous; filaments 0.5-0.7 mm long; young filaments and anthers purplish. Ovary ellipsoid and smaller than its prominent style; style ellipsoid to obovoid, 0.5-0.6 mm long, hyaline glandular-dotted, purplish when young; stigma terminal, flat, covering most of large, abaxially inclined, stigmatal plateau of 0.4-0.5(-0.6) mm diam. Fruits ellipsoid, 0.8-1.0 x 0.7-0.8 cm, pedicellate; pericarp granulose when young, slightly verruculose when maturing; style conical.

Taxonomic summary

Additional specimen examined. MEXICO. México: distr. Temascaltepec, mun. Luvianos, Oak woods, 8 Aug. 1933, *G. Hinton 4494* (ILL!, K [2]!, NY!, US!).

Etymology. The specific epithet refers to the inflorescences with relatively short rachis but distinctly long peduncle.

Distribution and habitat. Both known collections are from the Mexican municipality of Luvianos, where the species grows at the elevation of approximately 1,500 m in oak forest on rock walls.

Phenology. The plant has been found above-ground in July and August, flowering and fruiting.

Remarks

The differences between this species and *Peperomia cavispicata* are given under that species. *Peperomia elatior* shows a pistil morphology resembling that of *P. ampla* and *P. cavispicata*. The prominent style is obovoid in *P. elatior*, ovoid in *P. cavispicata* and cylindrical in *P. ampla*. *Peperomia elatior* is distinct in its flat stigma covering the terminal stigmatal plateau, the latter larger than in both other species. *Peperomia elatior* is also distinct in its pedicellate fruits.

Note. Trelease already recognized *Hinton 4494* as representing a new species. He annotated the ILL specimen with an epithet honoring Hinton as the type but never published the name. The name *P. hintonii* Yunck. was later published for a different species (Yuncker, 1965: 415). Yuncker apparently never saw the ILL specimen annotated by Trelease but did annotate the US duplicate: “This seems unique in its thick, fleshy spikes. Have been unable to match it and is possibly undescribed”.

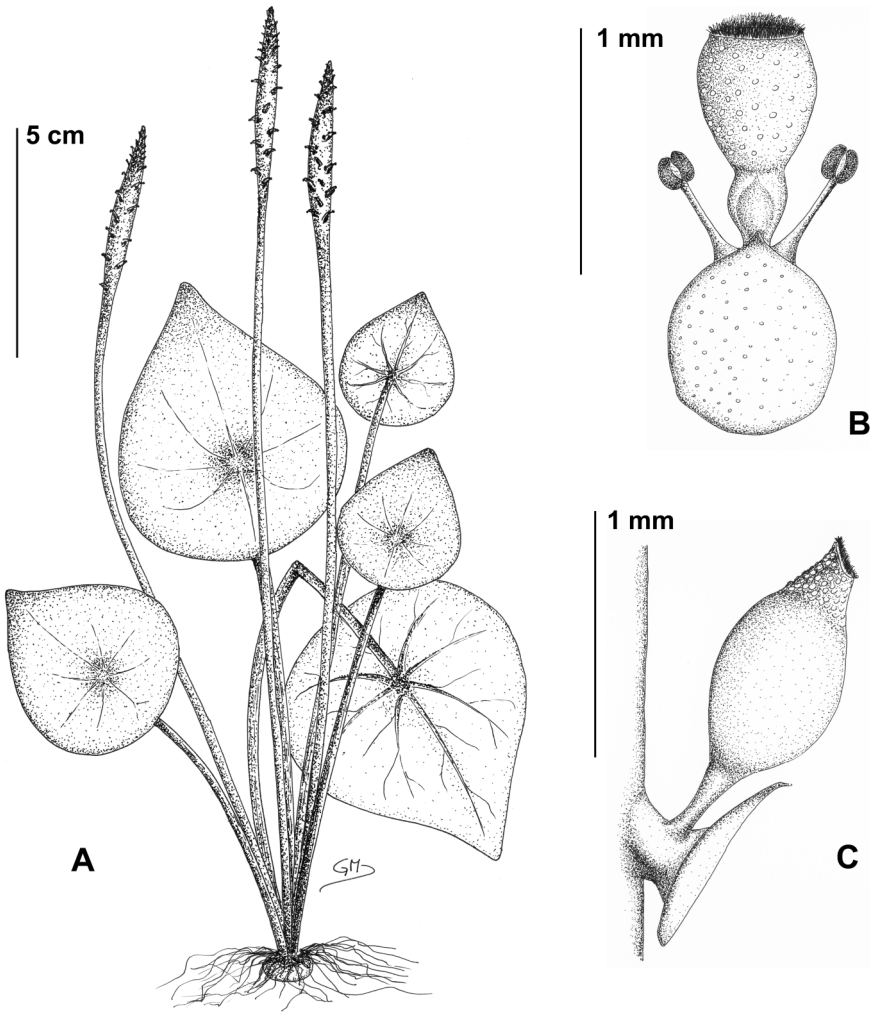


Figure 7. ***Peperomia elatior*. A, general habit. B, flower (frontal view). C, fruit (lateral view).**

6. *Peperomia exclamationis* G.Mathieu, n. sp. (Fig. 8)

Type: MEXICO. Michoacán: along road Coalcoman – Aquila, 18°41'20.1"N, 103°24'44.6"W, 1,260 m, 25 Jul. 2007, *M-S. Samain et al. 2007-045* (holotype: BR!; isotypes: GENT!, MEXU!, MO!).

A Peperomia questionis *G. Mathieu differt rachidi erecta longioreque, pedunculo breviore, lamina angustiore longioreque, apice longe acuminata, bacca ellipsoidea pedicellata, pericarpio non papillato vel basaliter minute papillato, bractea florali non glanduloso-punctata.*

Delicate herb, up to 5 cm tall in vegetative stage, entirely glabrous. Tuber globose, to 5 mm diam., yellow, smooth, rooting at the apex. Leaves 1-5 per plant; petiole 3-5 cm long; lamina membranous, drying translucent, deltoid, 2-6 x 1-4 cm, length/width ratio 1.5-2, the apex acute to acuminate, the base truncate to rounded, rarely subcordate, peltate 1-5 mm from base, 5-7-palmatinerved. Spadices 1-10 per plant; peduncle slender, 4-6 cm; rachis slender, erect, distal 1 cm sometimes bent, exceptionally split, (3-)4-8(-9) cm, longer than petiole except in small inflorescences, rather loosely white flowered. Floral bracts 0.4-0.5 mm diam., elliptic, peltate, the apex acute to acuminate. Stamens early deciduous; filaments 0.1-0.2 mm long; anthers ca. 0.2 mm long. Ovary ellipsoid; stigma terminal, cone-shaped on small stigmatal plateau. Fruits narrowly ellipsoid, 0.6 x 0.3 mm, together with floral bract on top of firm, broad conical and tapering pedicel; pericarp granulose or minutely papillate at base; style long conical to cylindrical.

Taxonomic summary

Etymology. The specific epithet refers to the erect rachis by which the species is easily distinguished from *P. questionis* G.Mathieu.

Distribution and habitat. *P. exclamationis* is only known from its type collection, made in tropical deciduous forest in the state of Michoacán. It was found at an elevation of 1,260 m in a shady environment, growing on vertical rock walls together with mosses and small ferns in a thin substrate layer.

Phenology. The plant has been found above-ground in July, flowering and fruiting.

Remarks

Peperomia exclamationis has to be distinguished from *P. questionis* G.Mathieu with which it shares peltate deltoid leaves. Although its distal end may be bent, the rachis of *P. exclamationis* is distinctly straighter, lacking the typical curl of almost the entire rachis that led to the choice of the epithet in *P. questionis*. This difference is obvious when comparing living plants of both species but might be less evident in herbarium specimens. The length/width ratio of the leaves of *P. exclamationis* is commonly 1.5-2 whereas in *P. questionis* it is about 1. Unlike *P. questionis*, the rachis is usually longer than the peduncle in *P. exclamationis.* In the latter species, the leaves are also long-acuminate whereas they are short-acuminate in *P. questionis*, resulting together with the different length/width ratio in a distinctly different leaf shape. Additionally, both species also differ in their fruit morphology: ellipsoid, pedicellate, with the pericarp not papillate or minutely papillate at the base in *P. exclamationis* versus oblong, sessile, with the pericarp entirely and distinctly papillate in *P. questionis*.

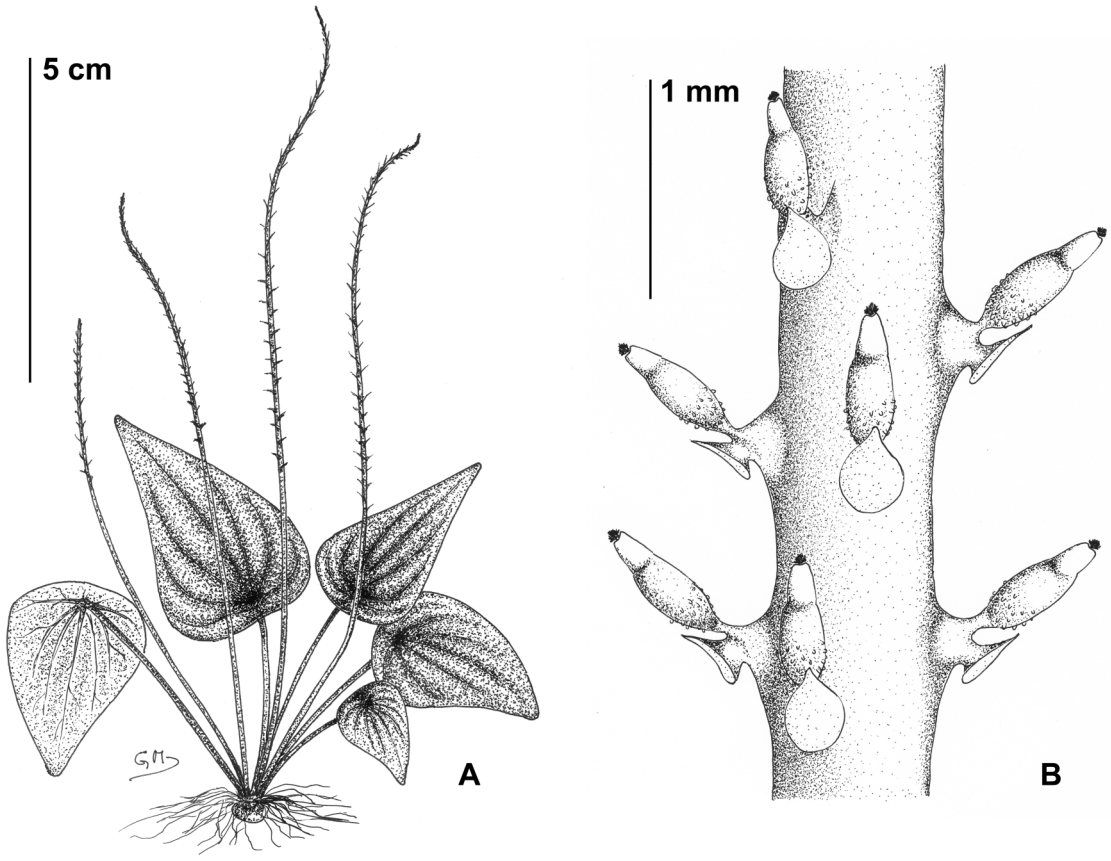


Figure 8. ***Peperomia exclamationis*. A, general habit. B, part of rachis.**

7. *Peperomia occulta* G.Mathieu, n. sp. (Fig. 9)

Type: MEXICO. Chiapas: mun. Oxchuc, 15 km N of Tenejapa, paraje Koltol'te, "Sumidero Yochib", 16°51'42.9"N, 92°27'13.9"W, 1,320 m, 6 Aug. 2007, *M-S. Samain et al. 2007-088* (holotype: BR! isotypes: GENT!, K!, MEXU!, MO!, P!, US!).

A Peperomia gracillima *S. Watson differt lamina peltata subcentrali, inflorescentia filiformiore, bacca longe ellipsoidea, pedicellata, longe stylifera.*

Delicate herb up, to 2 cm tall in vegetative stage, entirely glabrous. Tuber globose, to 3 mm diam., the surface yellow, smooth, rooting at the apex. Leaves 1-10 per plant; petiole 1-4 cm long; lamina membranous, translucent, irregularly orbicular, 0.7-2.5 cm diam., the apex usually rounded, sometimes obtuse, peltate at ca. 1/3 of leaf length from base, with 7 main nerves faintly visible. Spadices 1-5 per plant; peduncle filiform, 3-5 cm long; rachis filiform, (2-)5-10(-12) cm x 0.5 mm, loosely flowered. Floral bracts elliptic, 0.5 x 0.3 mm, the apex acute, centrally peltate, hyaline glandular-dotted. Stamens with filiform filaments, 0.2-0.3 mm long; anthers minute, smaller than 0.1 mm. Ovary ellipsoid; stigma terminal, globose, fimbriate. Fruits long ellipsoid to cylindric; body 0.6-0.7 x 0.2-0.3 mm, pedicellate; pericarp granulose; style 0.4-0.5 mm long, papillate.

Taxonomic summary

Additional specimens examined. MEXICO. Oaxaca: mun. Valle Nacional, along highway 175 between Valle Nacional and Ixtlan, 17°36'07.6"N, 96°22'44.0"W, 1,635 m, 9 Aug. 2007, *M-S. Samain et al. 2007-095* (BR!, GENT!, MEXU!); loc. cit., 17°35'15.1"N, 96°29'21.8"W, 2,265 m, 9 Aug. 2007, *M-S. Samain et al. 2007-098* (MEXU!); Veracruz: mun. Uxpanapa, Ejido Álvaro Obregon, road La Chinantla – Nuevo Acapulco, ojo de agua 'El Resumidero', 17°19'N 94°24'W, 70 m, 7 Jan 2010, *T. Krömer et al. 3873* (BR !, CITRO).

Etymology. The specific epithet refers to the habitat where the type collection was made, hidden in the deep shade of a cave entrance.

Distribution and habitat. *Peperomia occulta* is reported from the Mexican states of Chiapas and Oaxaca where it occurs in cloud forest (bosque mesófilo de montaña *sensu* Rzedowski, 2006) at an elevation between 1,320-2,265 m. Additionally, the species has also been collected in Veracruz where it occurs at a significantly lower elevation (70 m). At the type locality in Chiapas, plants grow on wet rock walls in a loamy substrate. At the same spot, but more exposed to daylight, occurs *Peperomia turckheimii* C.DC. The other collections were growing, together with mosses, on wet rocks close to a small waterfall in tropical rainforest. Although quite shady, their locations are more exposed to daylight than that of the type.

Phenology. The plant has been found above-ground in August, flowering and fruiting.

Remarks

The thin, translucent leaves, short petioles and long threadlike inflorescences together with the shape of the fruits make *P. occulta* easily distinguishable from other small geophytic species. The extremely dark habitat where the type collection was made suggests that this environmental element may also be helpful in identifying the species. *Peperomia gracillima* is also a delicate geophyte with thin, translucent leaves bound to rather shady habitats. It is a rather rare species and its name is often misapplied to other geophytic *Peperomia* species. According to its type collection from Jalisco (*Palmer 585* - BM!, F, G!, GH!, K!, MEXU!, MO!, NY!, P!, PH!, UC!, US!) it also has rather threadlike inflorescences, although less delicate than in *P. occulta*. However, *P. gracillima* has larger tubers (to 1 cm diam) and shows leaves that are centrally peltate and have a perimarginal nerve, distinctly visible on the translucent leaves. In *P. gracillima* the fruits are (sub)globose, sessile and have a short style. In *P. occulta* the fruits are narrowly ellipsoid, pedicellate and have a long style. The fruits of *P. occulta* resemble those of *P. mexicana* (Miq.) Miq.

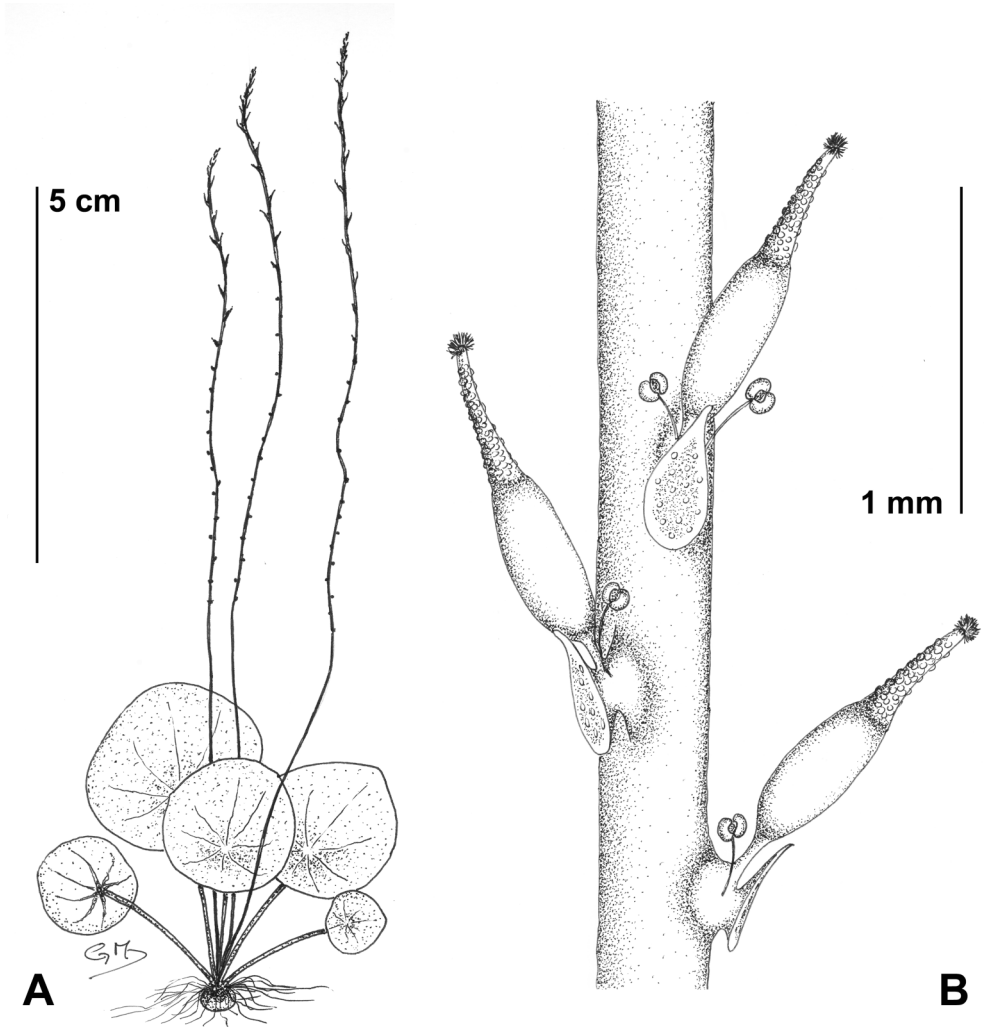


Figure 9. ***Peperomia occulta*. A, general habit. B, part of rachis.**

8. *Peperomia paramuna* Callejas, n. sp. (Fig. 10)

Type: COSTA RICA. San José: Cantón de Pérez Zeledón, P.N. Chirripó, trail from "Centro Ambientalista el Páramo" to "Sabana de Los Leones", 9°26'15"N, 83°30'36"W, 3,100-3,400 m, 18 Jul. 1998, *E. Alfaro 1767* (holotype: INB!; isotype: MO!).

A Peperomia pedicellata *Dahlst. differt inflorescentia 0.4-15 mm nec 20-40 mm longa, filamento minore 0.2 mm nec 0.4-0.8 mm longo, fructu globoso nec fusiformi.*

Delicate herb, entirely glabrous. Tuber globose, 5-10 mm diam, brownish and rugulose when dry, rooting at apex. Leaves 4-8 per plant; petiole 0.6-3.2 cm long, thin and fragile when dry; lamina orbicular to widely ovate (4-)13-30 mm diam., the apex rounded to obtuse, rarely short-apiculate, the base rounded, 7-palmatinerved, with main nerves diverging from base at angles of 95°- 45°, branched to sides, the secondary and tertiary nervation scarcely visible and opaque, evident only abaxially, the main nerve slightly raised abaxially, the lamina thick and almost fleshy and green when living, thick brownish and rigid when dry. Spadices 1-10 per plant, erect and white when living, rigid and brownish when dry; peduncle rigid when dry, 2.5-10 cm long, rachis short compared to the peduncle, 0.4-15 mm x 0.7-1 mm, rigid when dry, flowers sessile, loosely arranged on the rachis, widely spaced when in fruit. Floral bracts orbicular, the apex acuminate, folded on the sides when dry, appearing almost elliptic, prominent (as compared to the flowers), 0.6-1.0 mm diam., thick at the center, marginally membranous. Stamens with short filaments, 0.2 mm long; anthers prominent, 0.1-0.2 mm long. Ovary ovate, slightly distant from the rachis; stigma sessile, terminal. Fruit globose to ovate, 0.8 x 0.4-0.5 mm, short-apiculate at the apex; basally fixed and slightly distant and free from the rachis; pericarp slightly verruculose.

Taxonomic summary

Etymology. The specific epithet refers to the habitat of this species: open grasslands of paramo-like vegetation.

Distribution and habitat. Apparently restricted to open grasslands in a paramo region of Valle de los Leones, a locality in the National Park of Chirripó, at 3,100-3,400 m, on the Central Cordillera. We suspect the species is more common than reported. Likely the small size of the plants makes it difficult to locate this distinctive species.

Phenology. The plant was found above-ground in July, flowering and fruiting.

Remarks

Peperomia paramuna is easily distinguished from *P. gracillima*, erroneously reported for Costa Rica (Burger, 1971: 38), by its different habitat preference. *Peperomia paramuna* occurs in open grasslands whereas *P. gracillima* is found in deep shady canyons and at cave entrances. Furthermore *P. gracillima* has thin leaves that often dry almost translucent in contrast to the thick leaves in dry material of *P. paramuna*. The species exhibits some similarity with *P. pedicellata* Dahlst., but is easily distinguished by its globose to ovoid fruits and the much shorter filaments, 0.2 mm instead of 0.9 mm. *Peperomia gracillima* is probably restricted to Mexico whereas *P. pedicellata* occurs in southern Mexico, Guatemala, Honduras and El Salvador.

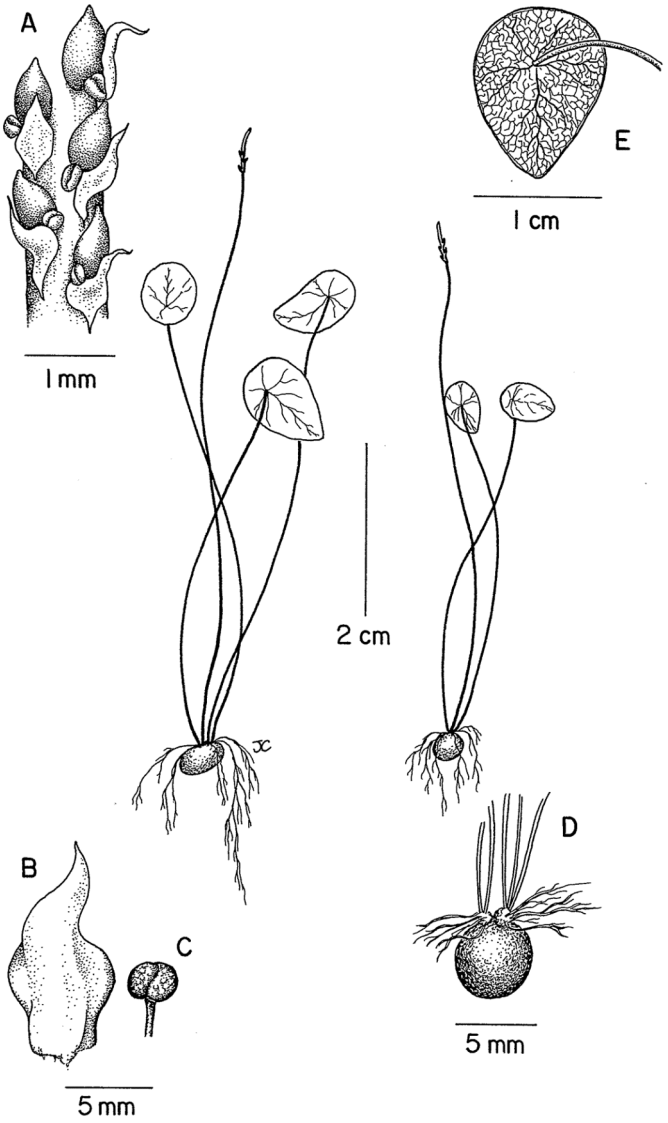


Figure 10. ***Peperomia paramuna*. General habit of two individual plants (center). A, part of rachis (fruiting). B, floral bract. C, anther. D, tuber. E, leaf nervation (abaxially).**

9. *Peperomia putlaensis* G.Mathieu**,** n. sp. (Fig. 11)

Type: MEXICO. Oaxaca: mun. Putla, 52 km SW of Tlaxiaco along the road Tlaxiaco – Pinotepa Nacional, Barranca de la Cama, 17°11'57.6"N, 97°53'17.2"W, 2,015 m, 10 Aug. 2007, *M-S. Samain et al. 2007-104* (holotype: BR!; isotypes: GENT!, K!, MEXU!, MO!, US!).

A Peperomia mexicana *(Miq.) Miq. differt lamina deltoidea non peltata et bacca breviter stylifera.*

Delicate herb, up to 10 cm tall in vegetative stage, stemless, entirely glabrous. Tuber globose, to 1 cm diam, brown, rooting at apex. Leaves 1-8(-10) per plant; petiole 2-10 cm, usually red spotted; lamina membranous, deltoid, to 5 x 4 cm, length/width ratio 1.1-1.6, the apex acuminate, acute to rounded at the tip, the base truncate or subcordate, rarely distinctly cordate, 5-7-nerved, the outermost nerves branching near their base, entirely green or red along nerves abaxially. Spadices 1-9 per plant; peduncle usually slightly red-spotted, to 4 cm long; rachis to 10 cm long, slender, erect, loosely flowered. Floral bracts elliptic, 0.6 x 0.4 mm, the apex acuminate, caudate in young bracts, centrally peltate, glandular-dotted. Stamens with short filaments; anthers 0.2-0.3 mm long. Ovary long-ellipsoid; style conical, 0.1-0.2 mm long; stigma terminal. Fruits oblong, 1.0 x 0.35 mm, short-pedicellate 0.1-0.2 mm; pericarp granulose, greenish beige to light brown.

Additional specimens examined. MEXICO. Oaxaca, Barranca del Pájaro, 9 Jun. 1985, *A. García Mendoza & R. Torres 1487* (MEXU [#458712, #458708]!); mun. Putla, 65 km SW of Tlaxiaco along the road Tlaxiaco – Pinotepa Nacional, Barranca del Pájaro, 17°07'51.1"N, 97°52'20.2"W, 1,315 m, 10 Aug. 2007*, M-S. Samain et al. 2007-105* (BR!, GENT!, MEXU!, MICH!, P!); 52 km SW of Tlaxiaco on the road Guerrero – Putla, 2,050 m, 18 Sep. 1993, *S. Zamudio & E. Pérez 9228* (BR!, IEB!, K!).

Taxonomic summary

Etymology. The specific epithet refers to the town of Putla in southwestern Oaxaca. So far, the species has only been collected in this area.

Distribution and habitat. All known collections are from a restricted area with oak forest between Santa María Tlaxiaco and Putla in the Mexican state of Oaxaca. The species occurs at 1300-2050 m, where it grows on wet rock walls in little substrate.

Phenology. The species is above-ground at least from June to September.

Remarks

By its non-peltate leaves, *P. putlaensis* can easily be distinguished from other geophytic *Peperomia* species with deltoid leaves like *P. exclamationis* G.Mathieu and *P. questionis* G.Mathieu. The leaves of *P. claytonioides* Kunth and *P. pedicellata* Dahlst., the latter often identified with its synonym *P. peltata* C.DC. (Mathieu and Callejas, 2006: 342), are usually more obovate than deltoid and also these species show peltate leaves. The subpeltate leaves of *P. mexicana* (Miq.) Miq., are usually distinctly longer (length/width ratio 2). Additionally, the fruits of the latter species are characterized by their long style.

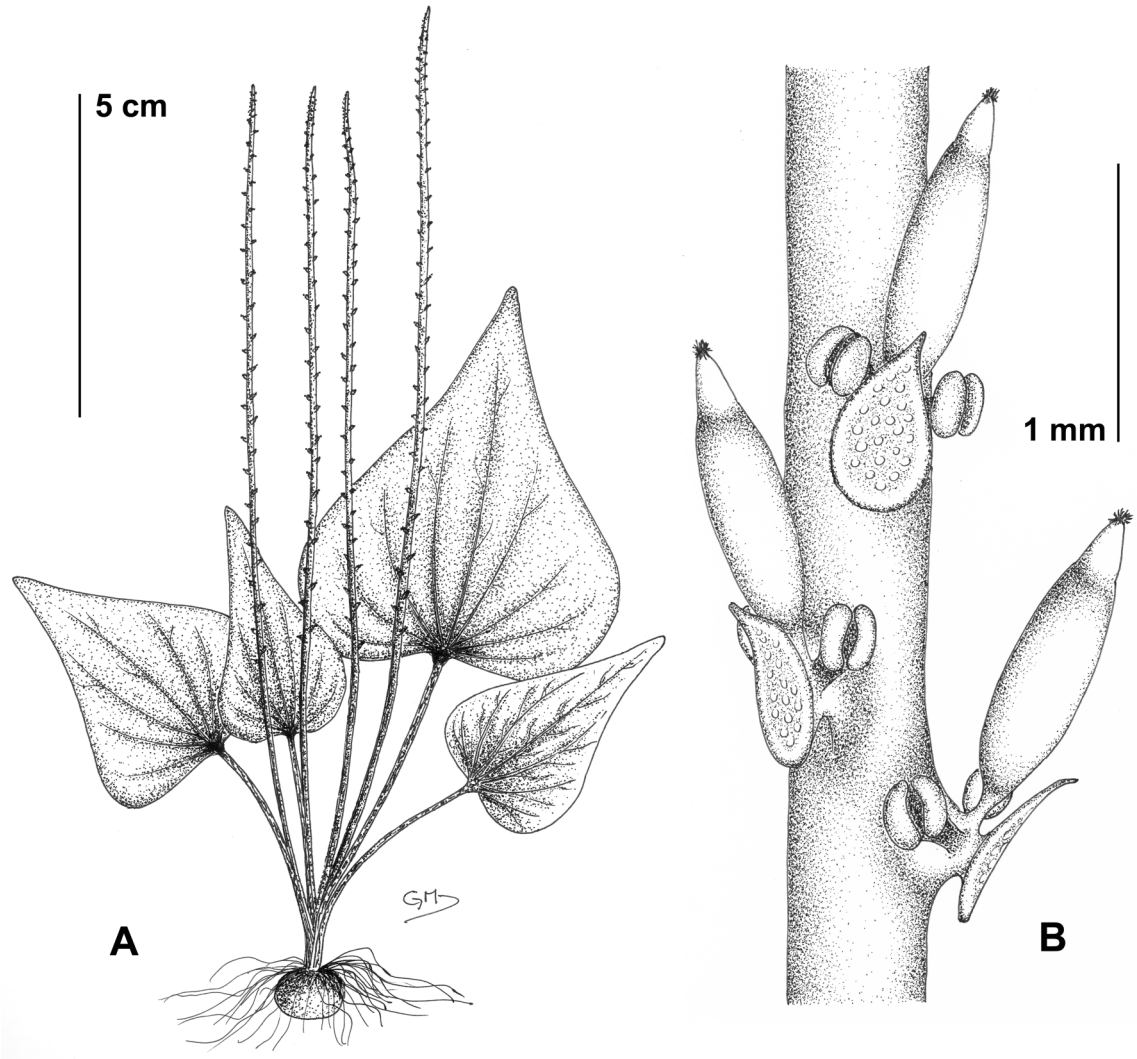


Figure 11. ***Peperomia putlaensis*. A, general habit. B, part of rachis.**

10. *Peperomia questionis* G.Mathieu, n. sp. (Figs. 12,13)

Type: MEXICO. Guerrero: along road Chilpancingo – Tepecichotlan, 17°29'00.2"N, 99°25'45.2"W, 1,060 m, 13 Jul. 2007, *M-S. Samain et al. 2007-014* (holotype: BR!; isotypes: GENT!, MEXU!).

A Peperomia exclamationis *G.Mathieu differt rachidi curvata brevioreque, pedunculo longiore, lamina latiore brevioreque, apice breviter acuminata, bacca oblonga sessili, pericarpio omnino papillato, bractea florali glanduloso-punctata.*

Delicate herb, up to 10 cm tall in vegetative stage, entirely glabrous. Tuber globose, to 1 cm diam., yellow, smooth, rooting at apex. Leaves 1-7(-9) per plant; petiole red, 2-10 cm long; lamina membranous, deltoid, 1.5-4.5 x 1.3-4.2 cm, dark green adaxially, whitish green abaxially, sometimes minutely but densely dark glandular-dotted combined with less dense whitish vesicles adaxially, the apex acute or acuminate, the base usually truncate or subcordate and peltate 1-5 mm from base, rarely deeply cordate and non-peltate, 5-7-palmatinerved, reticulately nerved in between, minutely papillate at main nerves adaxially, slightly depressed adaxially between main nerves. Spadices 1-7(-9) per plant; peduncle slender, 4-8 cm long, red; rachis 2-3 cm, erect only in juvenile stage when less than 1 cm, curled when maturing, white and loosely flowered. Floral bracts elliptic, 0.8 x 0.5 mm, centrally peltate, the apex long acuminate, white, dotted with large glands. Stamens with short filaments, 0.2-0.3(-0.4) mm long, deciduous when fruits are mature; anthers 0.2-0.3 mm. Ovary long-ellipsoid; surface papillate; style granulose; stigma terminal, globose, fimbriate; pistil white. Fruits oblong, the body 0.8-0.9 x 0.4 mm, sessile, the pericarp entirely long-papillate, though not always equally dense, young fruit white, mature fruit light brown; style conical, 0.2-0.3 mm long, shriveling and, in the mature fruit, somewhat abaxially inclined.

Taxonomic summary

Additional specimens examined. MEXICO. Guerrero: Petaquillas (5 km SE of Chilpancingo), road Petaquillas – Colotlipa, 4 km E of Petaquillas (by deviation), 20 Jul. 1977, *Germán et al. 520* (MEXU!); mun. Chilpancingo, Rincón de la Vía, 17°17'15"N, 99°28'55"W, 14 Jul. 1968, *Kruse 1858* (MEXU!); highway to Acapulco km 338, 3 km beyond Acahuizotla, 3,000 ft [915 m], 23 Aug. 1948, *H. Moore & C. Wood 4723* (BH!, GH!, MICH!, NY!).

Other record. MEXICO. Guerrero: mun. Chilpancingo, Hwy Chilpancingo-Acapulco, between km 38 and 39, 17°19'08"N, 99°28'10.3"W, 13 Jul. 2007, *M-S. Samain et al. 2007-s.n.* (photographs of plant *in situ*, GENT!).

Etymology. The specific epithet refers to the marked curl of the rachis, which makes the inflorescences resemble question marks and which seems to be a consistent feature of

this species in the wild. However, under greenhouse conditions, the rachis tends to become longer and decumbent. In that case, the characteristic curl may be less obvious or even absent.

Distribution and habitat. The species is reported from tropical deciduous forest in the state of Guerrero, growing in small soil pockets on shaded rocks and rock walls, elevation around 1,000 m.

Phenology. The species has been found above-ground in July and August.

Remarks

The differences between this species and *Peperomia exclamationis* are given under that species. *Peperomia questionis* differs from *P. putlaensis* G.Mathieu, which has non-peltate leaves and has peduncles shorter than the rachis. In *P. questionis* the fruits are sessile whereas they have a long pedicel and a short style in *P. pedicellata* Dahlst. In *P. questionis* the length/width ratio of the leaves is commonly about 1 whereas it is 1.5-2 in *P. exclamationis*.

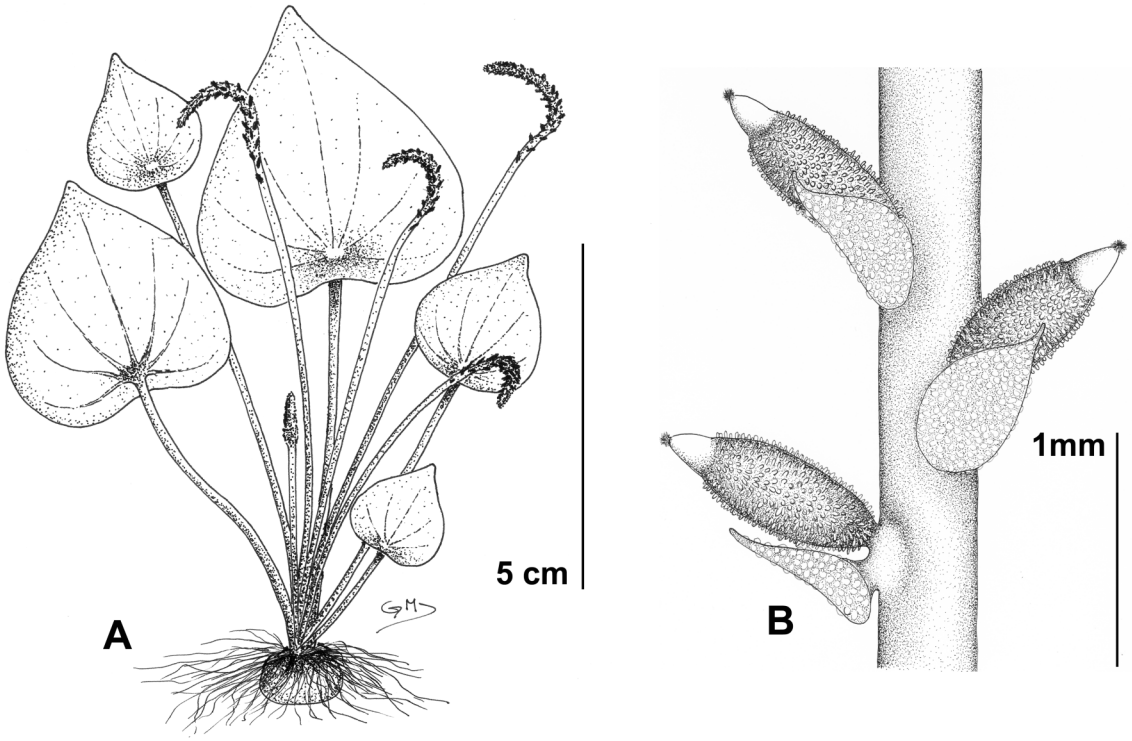


Figure 12. ***Peperomia questionis*. A, general habit. B, part of fruiting rachis.**

Figure 13. ***Peperomia questionis*** **in situ.**

11. *Peperomia tepoztecoana* G.Mathieu, n. sp. (Fig. 14)

Type: MEXICO. Morelos: Sierra de Tepoztlán, 6 May 1900, *Pringle 9047* (holotype: US!; isotypes: GH!, MICH!, VT!)

A Peperomia chutanka *Pino differt lamina ovata, utrinque viridi, petiolo basi latiore, pericarpio reticulato, praeferit locos humidos umbrososque.*

Robust herb, up to 25 cm tall in vegetative stage, entirely glabrous. Tuber to 7 cm long and 3 cm diam., irregular, brown, rooting at base, with several contorted trunk-like outgrowths at tuber apex, sometimes branching, grey-brown, to 18 cm long and 1 cm diam., with distinct distal petiole scars, the leaves and inflorescences at apex or body of trunks, less commonly at growing points at apical side of tuber. Leaves 1-10 per plant; petiole 5-10 cm x 2-4 mm, usually basally widest; lamina chartaceous, ovate, rarely almost orbicular, to 10 x 7 cm, length/width ratio (1-)1.3-1.4, peltate at 1/4 to 1/3 of leaf length from base, the apex obtuse to acute or slightly acuminate, the base rounded, 10-palmatinerved, glossy green adaxially, slightly whitish green abaxially. Spadices 1-10 per plant; peduncle (1-)4-7 cm; rachis (5-)7-12 cm, moderate-densely flowered. Floral bracts orbicular to elliptic, 0.8-1.0 mm long, peltate, the apex acute to acuminate, glandular-dotted. Stamens with filaments 0.4-0.7 mm long; anthers ca. 0.4 mm long. Ovary long ellipsoid, narrower than style; stigma terminal. Fruits long-ellipsoid, 0.7-0.9 x 0.5-0.6 mm, shortly pedicellate; pericarp reticulate; style long- conical, almost half as long as the fruit body.

Taxonomic summary

Additional specimens examined. MEXICO. Morelos: near Cuernavaca, 7,000 ft, 2 Jun. 1898, *C. Pringle 7630* (GH!, VT [mounted together with *C. Pringle 5182*, which is *P. lanceolatopeltata* C.DC.]!); mun. Tepoztlán, 'El Tepozteco', 18°59'10.7"N, 99°06'07.7"W, 1,950 m, 19 Aug. 2007, *M-S. Samain et al. 2007-121* (BR!, GENT!, MEXU!).

Etymology. The specific epithet refers to "El Tepozteco", the pyramid above the town of Tepoztlán. All known collections are from a narrow canyon just below the pyramid. Apart from the type, the species is known from another Pringle collection. The label of *Pringle 7630* at VT (where Pringle's first sets are housed) mentions 'parque', by which the archeological park with the Tepozteco pyramid is meant. *Pringle 9047*, the type, collected two years later, is likely made at the same location. The steep path to the pyramid was, certainly in those days, probably the only access to the heights of the Sierra de Tepoztlán where *P. tepoztecoana* is growing.

Distribution and habitat. All known collections are from one locality. The species grows on shaded vertical rock walls.

Phenology. The plant produces leaves at least between May and August. In August it is no longer flowering or fruiting.

Remarks

The trunk-like outgrowths on top of the tuber are the most typical feature of this species. They resemble similar structures in *Peperomia chutanka* Pino from Peru, but in that species they tend to be more numerous and more branched. The leaves of *P. chutanka* are usually also a little smaller and rather orbicular to elliptic than ovate, and. especially differ in the violet abaxial side. The petiole in *P. tepoztecoana* is widest at the base whereas it is widest at the apex in *P. chutanka*. The reticulate fruit pericarp of *P. tepoztecoana* is not reported in *P. chutanka*. The latter species prefers exposed, dry rock walls where it occurs often together with lichens. *Peperomia tepoztecoana* prefers the shady and rather moist rock walls of deep canyons.

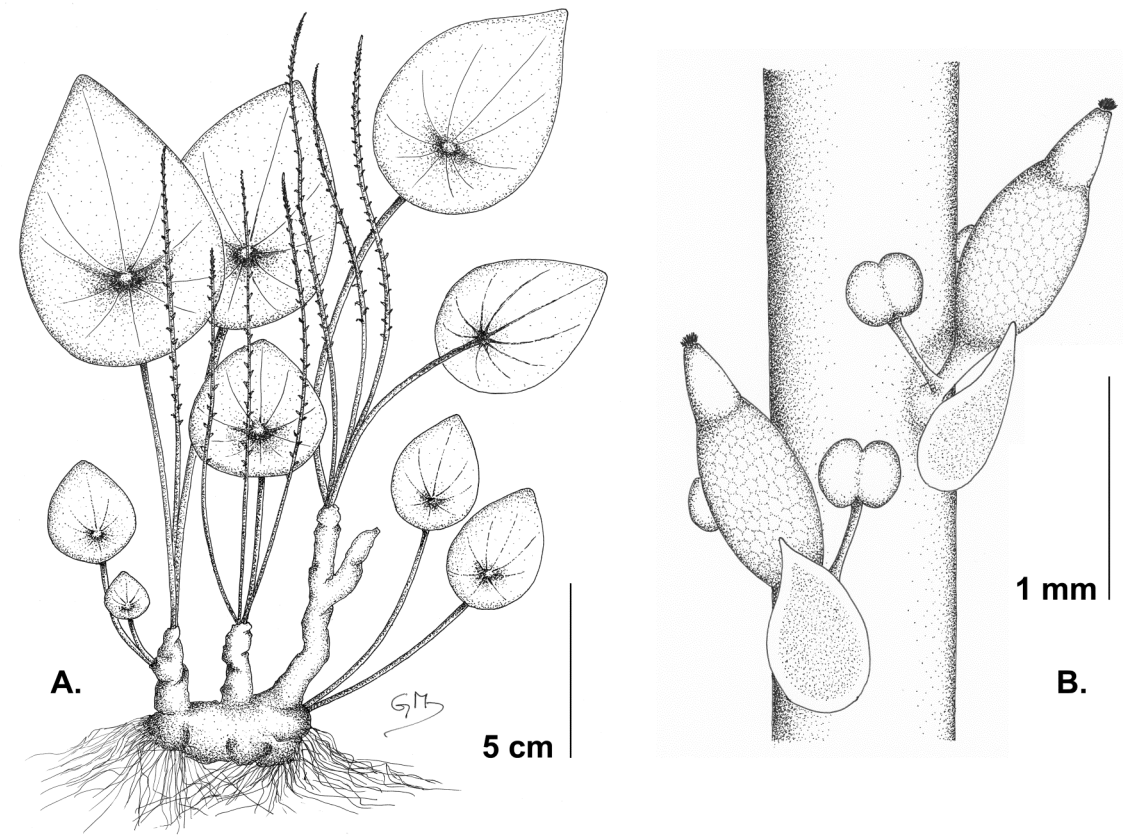


Figure 14. ***Peperomia tepoztecoana*. A, general habit. B, part of fruiting rachis.**

12. *Peperomia toledoana* Callejas, n. sp. (Fig. 15)

Type: BELIZE. Toledo: Columbia River Forest Reserve, Union Camp, ca. 3 km W of SW end of Little Quartz Ridge, creek bed with exposed limestone rock in area with semideciduous forest 35-30 m tall, 16°23'53"N, 89°07'34"W, 700-750 m, 22 Feb. 1997, *B. Holst 5959* (holotype: HUA!; isotypes: MO!, SEL!).

A Peperomia claytonioides *Kunth differt foliis nigro-puncticulatis, inflorescentia 0.3 mm nec 0.5 mm diameter.*

Delicate, lithophytic herb, entirely glabrous. Tuber ellipsoid, 5 mm long, 3 mm diam., rooting at the apex, without apparent foliar scars. Leaves 4-7 per plant; petiole 2.5-3.8(-5.1) cm long, slightly striate; lamina ovate to widely ovate or elliptic-ovate, peltate 2-5 mm from the margin, 1.2-2.4(-2.9) x 1.5-1.8 (-2.3) cm, the apex rounded, obtuse or short acute with obtuse tip, the base rounded to truncate or slightly cordate, 5-6-palmatinerved, the innermost nerves diverging in angles of 45°, outer nerves diverging in angles of 80°, the tertiary nervation evident abaxially, forming prominent areoles, these loops connected towards margin forming a prominent marginal nerve that extends at least 2/3 of leaf, dense but minutely pellucid-dotted, the idioblasts dark brownish to blackish in dry leaves, abundant and visible in both faces, green, membranous when living and dry. Inflorescences compound, the common axis of 5.5-12 cm bearing 2-6 spadices, one terminal, the others slightly lateral, sub-erect, white when living and dry, each individual spadix from axil of a small deltoid bract, 0.8 mm diam, membranous and persistent, the spadices slightly spaced by short internodes, 0.3-0.7 mm long; peduncle of each individual spadix 2-4 mm long; rachis 2-5 (-9) cm x 0.3 mm, filiform, white in dry material, the flowers densely congested on rachis at anthesis, widely spaced in fruit (0.7-1.2 mm). Floral bracts widely obovate, prominent, 0.6-1.1 mm diam, the base adnate and decurrent along the rachis, the lamina free from the rachis, expanded and wider, white and almost translucent. Stamens with filaments 0.4 mm long; anthers 0.3 mm long. Ovary oblong, 0.7 x 0.01 mm, sessile; stigma apical. Fruit globose, 0.4 mm diam., sessile and basally attached to rachis, pale brownish, apically long stylose, the stylar portion 0.3 mm long, and obliquely oriented to the body of the fruit,.

Taxonomic summary

Additional specimens examined. BELIZE. Toledo: Columbia Forest Reserve, Camp 2, 6-8 km SE of Union Camp, trail from camp 2 to camp 3, moist tropical mixed hardwood forest with palm and shrub understory, soils derived from limestone, 16°23'14"N, 89°4'37"W, 665-700 m, 15 Feb 1997, *T. Hawkins 1424* (HUA!, MO!, SEL); Southwestern Maya Mountains, Columbia River Forest Reserve, Union Camp, ca 3 km W of SW end of Little Quartz Ridge, semi-evergreen forest on undulating limestone hills, areas harvested for

mahogany and chicle in the past, otherwise undisturbed, on mossy rocks, 16°23'N, 89°09'W, 700-750 m, 4 Apr. 1992, *B. Holst 3882* (HUA!, MO!, NY, SEL).

Etymology. The specific epithet is derived from the type locality in the department of Toledo, Belize.

Distribution and habitat. The species grows on limestone blocks of small streams and apparently is locally abundant in forests dominated by *Terminalia amazonia* and *Manilkara zapota*.

Phenology. The species has been found above-ground in July.

Remarks

At first sight *Peperomia toledoana* looks similar to *P. claytonioides* Kunth. However, *P. toledoana* is a more fragile plant, with much thinner inflorescences; its leaves are 1.2-2.4(-2.9) x 1.5-1.8(-2.3) cm and the fruiting rachis 2-2.4(-2.9) x 0.3 mm whereas in *P. claytonioides* leaves are (2.7-)3.5-8.7(-12.5) x (1.1-)1.7-4 cm and the fruiting rachis (3-)5-8 x 0.5 cm. The leaves in *P. toledoana* are black pellucid dotted, whereas this is lacking in *P. claytonioides*.

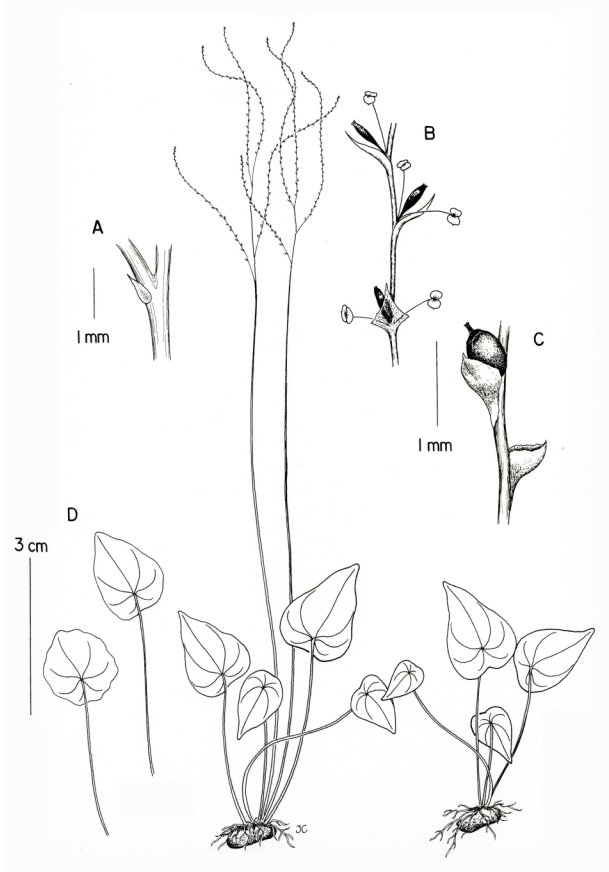


Figure 15. ***Peperomia toledoana*. General habit, flowering (center) vegetative (bottom right). A, detail of the bract at the base of each peduncle of a each individual spadix. B, part of rachis (flowering). C, part of rachis (fruiting). D, individual leaves, showing variation in the apex.**

13. *Peperomia unifoliata* Callejas, n. sp. (Fig. 16)

Type: COSTA RICA. San José: canton Acosta, distr. Cangrejal, Hacienda Tiquires, Quebrada Ayarales, pluvial montane forest, 800-1,100 m., 9 Jul. 2003, *R. Callejas & F. Morales 13253* (holotype: INB!; isotype: HUA!).

A Peperomia linaresii *Véliz differt foliis peltatis, erectis, nigro-puncticulatis, inflorescenctia erecta, fructu pedicellato, breviter styloso 0.3 mm nec 0.9 mm.*

Minute epilithic, shade loving herb, entirely glabrous. Tuber globose-ovoid, greenish brown when living, 0.8-2.5 mm diam, with rather few elongated roots at apex of tuber. Leaves solitary; petioles erect, 0.7-1.5 cm long; not pellucid dotted, green when living, brownish and thin when dry, the lamina ovate to elliptic-ovate, peltate about 1-2 mm from the base 1.8-2.5(-3) x 1.3-2 cm, the apex slightly acute, the base rounded to truncate, 5-7-palmatinerved, the main nerves diverging from the base at angles 40°-85°, the nerves branched at the apex and loop-connected, the main nerve with a pair of secondary nerves diverging in the upper third portion, the tertiary nervation forming a dense areolate pattern, the nerves impressed in both faces, only the main nerve slightly elevated abaxially, the lamina green-metallic and membranous when living, thin, almost translucent and pale green when dry, the margin eciliate, densely and minutely pellucid-dotted, the idioblasts blackish, abundant and visible adaxially, opaque abaxially. Spadix solitary, apparently not axillary to the leaf but lateral, erect to slightly curved at the apex, white at anthesis, greenish at fruit; peduncle 2.5-4 cm x 0.2 mm, filiform, pale green when living; rachis 1-1.5 cm x 0.1 mm at anthesis, 2-3.5 cm x 0.1 mm in fruit, striate and whitish, almost translucent when dry. Floral bract orbicular to ovate when dry, 0.2-0.3 mm, subsessile and appearing free from the rachis when dry, the flowers congested at anthesis, widely spaced in fruit. Stamens with long filaments, 0.7-0.9 mm long; anthers minute, 0.1 mm. Ovary subsessile and free from the rachis, ovoid to ellipsoid and short-stylose; stigma terminal. Fruit ellipsoid, the fruit body 0.6-0.7 mm long, 0.2-0.3 mm pedicellate, the pedicel rigid, the pericarp pale green to brownish, long-stylose, the stylar portion 0.3 mm long.

Taxonomic summary

Etymology. The epithet refers to the single solitary leaf of each individual plant.

Distribution and habitat. The type collection was found growing on exposed and moss covered walls of clay-granitic soils along a road in a pluvial montane forest region. Due to the size of the plants and the fact that each one has a single leaf, collecting of the species was rather accidental and it took some time before we were able to recognize the species among the dense and wet mats of mosses, hepatics and small ferns.

Phenology. The plant has been found above ground in July.

Remarks

Peperomia linaresii Véliz, recently described from Guatemala and El Salvador (Véliz, 2007: 10), also has 1(-2) leaves per plant and a solitary inflorescence. Both species can be distinguished by the pedicellate fruits with short style of *Peperomia unifoliata* in contrast to the sessile and long-stylose fruits of *P. linaresii*. Furthermore the leaves in *P. linaresii* are lanceolate to ovate and without any glands whereas those in *P. unifoliata* are elliptic-ovate and densely black pellucid dotted. The fusiform and pedicellate fruits of *P. unifoliata* are similar to those of *Peperomia pedicellata* Dahlst., but in *P. unifoliata* the pedicel is distinctly shorter (0.3 mm) than in *P. pedicellata* (0.4-0.6 mm) and the fruits are smaller (0.6-0.7 mm, not 0.8-1.2 mm long).

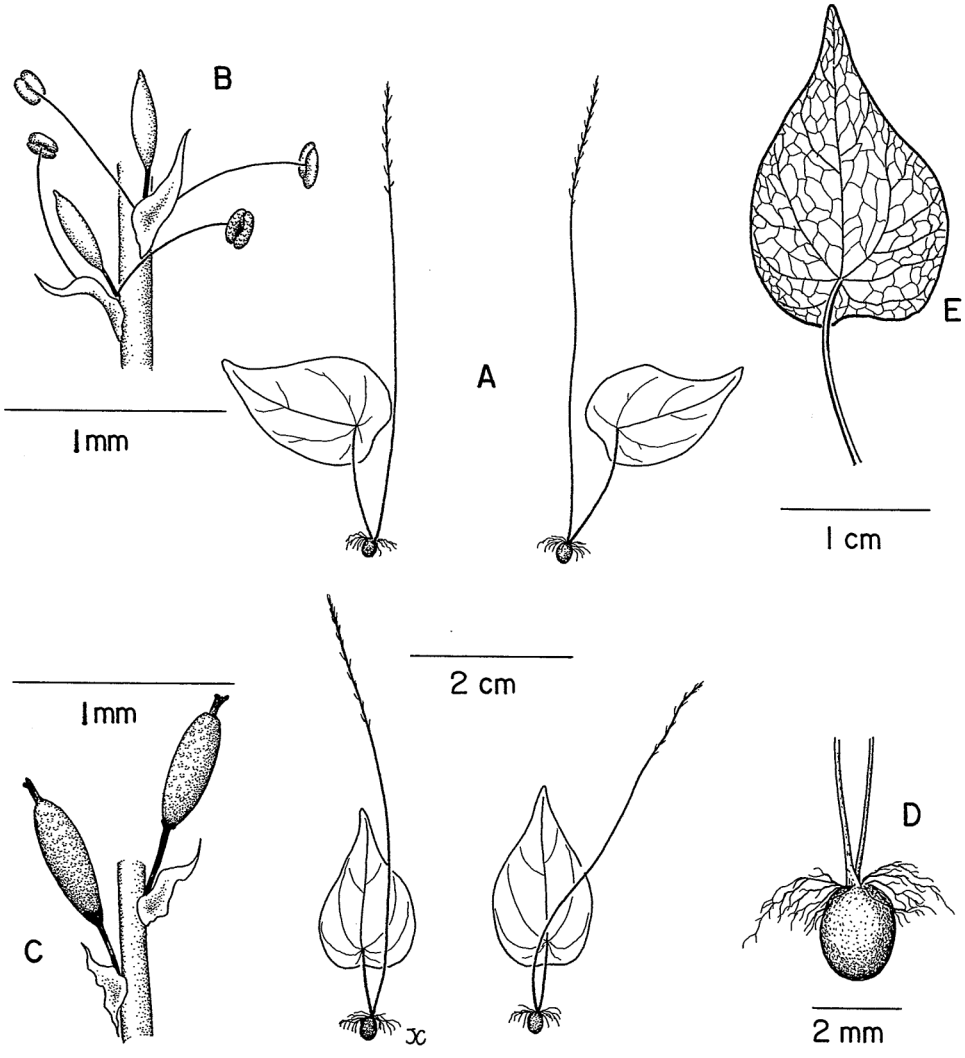


Figure 16. ***Peperomia unifoliata*. A, general habit, lateral (above) from behind (below). B, part of rachis (flowering). C, idem (fruiting). D, tuber. E, leaf nervation (abaxially).**

Acknowledgements

We thank the Secretaría de Medio Ambiente y Recursos Naturales (SEMARNAT; permit numbers SGPA/DGGFS/712/1397/07, SGPA/DGGFS/712/2486/09 and SGPA/DGGFS/712/0424/10) for permission to collect material; the staff of the Herbario Nacional and the Instituto de Bíología of the Universidad Nacional Autónoma de México for support with field and herbarium work; Hilda Arreola, Álvaro Campos, Rosamund Coates, Emmanuelle De Foy, Daniel De León, Hilda Flores, Abel García, Socorro González, Carolina Granados, Thorsten Krömer, Esteban Martínez, Marco Martínez, Juan Mendoza, Helga Ochotorena, Yocupitzia Ramírez, Alberto Reyes, Jerzy Rzedowski, Alfredo Ruíz, Gerardo Salazar, Victoria Sosa, Victor Steinmann and Sergio Zamudio for assistance with field work and logistics; Gerrit Davidse, Kanchi Gandhi and John Wiersema for their input on nomenclatural matters; Guillermo Pino, Michael Nee and Craig Swafford for constructive comments on the manuscript; the Botanical Gardens of Ghent University and Dresden Technical University for general support; and the curators of the herbaria at AAU, B, BH, BIGU, BM, BOLV, BR, C, CIB, CIIDIR, CM, CORU, CPUN, E, EBT, F, G, GB, GENT, GH, GOET, HEID, HUA, HUT, IBUG, IEB, ILL, INB, ISC, JE, K, L, LPB, LY, M, MA, MEXU, MICH, MO, NY, P, PH, PR, PRC, QCNE, S, SEL, TCD, U, UC, UPS, US, USM, VT, W, XAL and ZSS for the loan and permission to study material. This work was supported by the Research Foundation Flanders (FWO-Vlaanderen; FWO G.0172.07; FWO travel grants to the first and last author; the German Science Foundation (DFG; NE681/5-1, DFG; NE681/5-2); the Department of Biology, Ghent University; the Friends of the Botanical Garden Ghent; the Gesellschaft der Freunde und Förderer der TU Dresden, and the German Academic Exchange Service (DAAD PPP Colombia).

CHAPTER 3

Biogeography of *Peperomia* subgenus *Tildenia*

This chapter is a modified version of:

Symmank, L., Samain, M-S., Smith, J.F., Pino, G., Stoll, A., Goetghebeur, P., Neinhuis, C. & Wanke, S.

The extraordinary journey of *Peperomia* subgenus *Tildenia* (Piperaceae): insights into diversification and colonization patterns from its cradle in Peru to the Trans-Mexican Volcanic Belt. *Journal of Biogeography*, in press.

Summary

Aim

Peperomia subgenus *Tildenia* consists of approximately 60 species growing in seasonal habitats of Neotropical mountain areas from Mexico to Argentina. The subgenus can be split geographically, with almost equal diversity in the northern hemisphere (Mexico and Guatemala) and in the southern hemisphere (Peru and Bolivia). Only a few species are known from a limited number of localities between these two hotspots. As such, *Tildenia* is an ideal candidate to test time, direction and mode of migration of high mountain taxa against the background of the "Great American Biotic Interchange".

Location

The Andes with focus on the Central Andes, and the Mexican mountain chains, especially the Trans-Mexican Volcanic Belt.

Methods

To elucidate the spatio-temporal origin, subsequent colonisation and radiation of *Tildenia*, we combine Bayesian phylogenetics based on the chloroplast *trnK-matK-psbA* region, geo-referenced distribution data, fossil calibrated molecular dating approaches using both Penalized Likelihood and Relaxed Phylogenetics and Dispersal-Vicariance Analysis as well as Dispersal-Extinction-Cladogenesis for ancestral area reconstruction.

Results

Peperomia subgenus *Tildenia* is subdivided into six Andean clades and one Mexican and Central American clade originating from a north/central Peruvian ancestor. Molecular dating approaches converge on a stem age of approximately 38 MYA for *Tildenia* and a mostly Miocene diversification and colonisation.

Main conclusion

We detect a strong correlation between diversification of *Tildenia* and orogenetic events in the respective distribution centres. In the Andes, distribution was influenced by the Altiplano-Eastern Cordillera System as well as the Amotape-Huancabamba Zone, where the latter serves as both migration barrier and migration bridge for different clades. In contrast to most studies of high altitude taxa, we prove a south-north colonisation towards Central America and Mexico and confirm the latest view on the timing of the "Great American Biotic Interchange". In Mexico, the Trans-Mexican Volcanic Belt has played a major role in more recent radiations together with climatic oscillation and the formation of refugia.

Introduction

One of the major biogeographical issues of world biota is the distribution of flora and fauna between the two American landmasses that have been separated at least for most of the last 150 million years (e.g. McLoughlin, 2001). Isolation of the continents began with the rupture of supercontinent Pangaea into Laurasia (North America, Europe and Asia) and Gondwana (South America, Africa, Antarctica, Australia, Madagascar and India) in the late Jurassic (e.g. Torsvik & Van der Voo, 2002). The two American landmasses have been reconnected progressively by two comparatively small land bridges: the Isthmus of Tehuantepec since the late Miocene (Barrier *et al.*, 1998) and the Panama Isthmus since the middle Pliocene (Coates & Obando, 1996). Despite the long-lasting geographical separation, many plant and animal lineages show very close relationships between North and South America (e.g. Raven, 1963; Collins & Dubach, 2000; Moody *et al.*, 2001; Simpson *et al.*, 2005; Good-Avila *et al.*, 2006).

Since the beginning of biogeographic conceptual thinking, several hypotheses have been formulated to explain present-day distribution patterns between the two American continents:

I) A radiation took place before the rupture of supercontinent Pangaea in the Mid-Jurassic. However, earliest angiosperm lineages had just started diversification (e.g. Moore *et al.*, 2007; Magallón 2010).

II) Dispersal took place in a stepwise Cenozoic colonization of smaller land patches above sea level between the two American subcontinents (e.g. Gentry 1982; Pennington & Dick 2004). Suitable elevations for the 'island hopping' hypothesis might have been the "proto-Caribbean archipelago" reported from uncertain age between the Cretaceous and Eocene, the "proto-Greater Antilles" in the Middle and Late Eocene ca. 50 MYA or the "GAARlandia complex" at the Eocene-Oligocene boundary ca. 35 MYA (e.g. Gentry, 1982; Iturralde-Vinent & MacPhee 1999; Sanmartín & Ronquist, 2004). Furthermore, the presence of an island arc has been hypothesized to have occupied the current position of Panama about 15 MYA (Krzywinski *et al.*, 2001**;** Sanmartín & Ronquist, 2004).

III) There was a continuous migration of plant and animal groups via the Central American land bridge which was complete in the middle Pliocene (Keigwin, 1978; Coates & Obando, 1996; Coates *et al.*, 2003). The closure of the Panama Isthmus is generally seen as the initiation of a phenomenon known as the 'Great American (Biotic) Interchange' (Simpson, 1980; Stehli & Webb 1985). The exchange of Gondwanian and Laurasian derived biota between the two American subcontinents is seen as one of the keystones of Neotropical biodiversity (Burnham & Graham, 1999).

IV) Long distance dispersal (LDD) has shaped current distribution patterns independently of landmass position and migration barriers. This distribution mode requires functional adaptations for seed and fruit dispersal by wind or biotic vectors (e.g. Raven 1963; Vargas *et al.*, 1998).

Multiple paleobiological studies, mainly based on Neogene fossil evidence (summarised in Burnham & Graham, 1999), state a general south-north migration of tropical lowland plant groups between the two Americas, whereas a considerable amount of temperate and upland flora found today in South America has been suggested early on to be derived from Laurasian ancestors (Gentry, 1982). Recent molecular studies of Neotropical plant groups using molecular dating approaches largely confirm the forementioned migration patterns (e.g. von Hagen & Kadereit, 2003; Pennington *et al*., 2004, Bell & Donoghue, 2005; Hughes & Eastwood, 2006, Weigend *et al*., 2010).

The pantropically distributed giant genus *Peperomia* contains approximately 1650 species and has its main diversity in the Neotropics where it occurs in a wide range of habitats from sea level to above 4600 meters (Samain *et al.*, 2009). Despite its interesting distribution patterns at local, regional and global scales, comprehensive biogeographical studies are largely lacking. Raven & Axelrod (1974) speculated that Piperaceae may have had a Laurasian origin, perhaps reaching South America via Africa. Trelease (1930) proposed that *Peperomia* may be of South American origin, a hypothesis that accords with the study of Smith *et al.* (2008) showing that, based on molecular dating approaches and ancestral area reconstruction, *Peperomia* is of Neotropical origin.

The exclusively Neotropical *Peperomia* subgenus *Tildenia* (hereafter shortened as *Tildenia*) is one of the first diverging lineages within the genus (Wanke *et al.,* 2006; Samain *et al*., 2009). It contains approximately 60 species ranging from northern Argentina to northern Mexico in two diversity centres. The southern centre of diversity is found in the Andes of Bolivia and Peru whereas the northern one is located from central and southern Mexico to Guatemala (Hill, 1906, 1907; Mathieu *et al.*, in press; Samain *et al.*, in press).

The southern centre of diversity contains 29 species and extends from 7° S – 79°W in Peru to 21°S – 64°W in Bolivia. The species are known to occur up to approximately 4600 m above sea level with most of the species occurring between 2600 and 4000 m and rarely below 2000 m. The highest diversity is observed in the Peruvian department of Cajamarca and bordering areas of the departments of Amazonas and La Libertad (Samain *et al*., in press).

The northern centre of diversity contains 23 species and stretches from approximately 26° N – 106° W in Mexico to 15° N – 90° W longitude in Guatemala (Mathieu *et al.*, in press).

The Mexican and Central American species generally occur on volcanic soils between 1400 and 2600 m and rarely above 3500 m or below 500 m. Most of them occur in the Trans-Mexican Volcanic Belt (TVB, defined here following Ferrusquia-Villafranca 1993), the Central Plateau, and the Sierra Madre mountain system. The largest diversity of *Tildenia* species in Mexico is encountered in the TVB. Seventeen species of *Tildenia* are reported from this area, eight of which are endemics (Mathieu *et al.,* in press).

The two major diversity centres do not share any species. Ten species are known to occur between both diversity centres in Central America and northern South America, of which four are endemics (Véliz, 2007; Mathieu et al., in press). The remaining six species also occur in the northern diversity centre.

Tildenia species grow as geophytes in seasonal, mostly mountainous habitats with prominent wet and dry seasons (Hill, 1906; Mathieu *et al.*, in press, Samain *et al.*, in press). Different *Tildenia* species often occur in different microhabitats only some centimetres apart from each other, e.g. with one species growing exposed on large rocks and the other species in the crevices of the same rocks. All species are characterized by the presence of a perennial underground or partially aboveground tuber as an adaptation to seasonality. The flowering and fruiting period generally coincides with the local rainy season (Mathieu et al, in press; Samain *et al.*, in press). In contrast to most other lineages of *Peperomia* where the sticky fruits are hypothesized to be dispersed by means of zoochory (Haber, 2000, in Appendix 1, pp. 489-490), the fruits of *Tildenia* are usually not sticky and do not show obvious adaptations to any dispersal mechanism such as anemo- or zoochory. The life form and distribution pattern of *Tildenia* make this group an ideal candidate to test time, direction and mode of migration of high mountain taxa against the background of the “Great American Biotic Interchange”. The aims of this study are to 1) unravel the spatiotemporal origin of *Tildenia* and 2) trace the direction of colonisation and subsequent radiations that formed the two distinct diversity centers on both American continents. Therefore, we combine molecular phylogenetic analyses, geo-referenced distribution data, fossil-dated phylogeographic approaches and ancestral area reconstruction. Two competing hypotheses that shaped the current distribution pattern are tested in particular. The occurrence of *Tildenia* on both American continents constitutes a) an ancient process based on ‘island hopping’ or b) a younger continuous colonisation after the closure of the Panama Isthmus.

Materials and Methods

Sampling strategy

Forty-nine out of the approximately 60 known *Tildenia* species were sampled with 201 accessions for the present study, representing the entire distribution area. Nearly all species of both diversity hotspots were included as well as some accessions from immediately south of the northern hotspot. The outgroup consists of four representatives of Saururaceae (*Houttuynia*, *Gymnotheca*, *Saururus* (2 species)), one *Piper* species as well as three non-*Tildenia Peperomia* species. A list of accessions including voucher information, origin, locality data and GenBank accession numbers is provided in Appendix S1 as Supporting Information. DNA samples were primarily obtained from leaves collected in the field and dried in silica gel, complemented with material from herbarium specimens. Field work included not only recollection at known localities but also extensive exploration in large under-collected regions within the distribution area of *Tildenia* (Mathieu *et al.*, in press, Samain *et al.*, in press).

DNA-Isolation, amplification, and sequencing

Genomic DNA was extracted using a modified CTAB extraction (Liang & Hilu, 1996, Borsch *et al.* 2003). The chloroplast region, including the *trnK* intron, *matK* gene and *trnK-psbA*

spacer was amplified in two parts with an overlap for amplification and sequencing of 200 to 400 bp, depending on the primer set employed (see Appendix S2). Amplification included the following steps: 1) initial denaturation for 5 min at 94°C, 2) denaturation for 1 min at 95°C, 3) annealing for 1 min at 48°C, 4) elongation for 2 min at 68 °C, 5) 34 cycles of steps 2 to 4, followed by 6) 20 min at 68°C. The Polymerase Chain Reaction (PCR) was carried out as a 50 µl reaction, containing 1 µl DNA template of total genomic DNA (~ 10-50 ng, depending on quality), 10 µl dNTP mix (1.25 mM each), 2 µl of each primer (20 pmol/µl) and 1 µl Taq Polymerase (PeqLab). Amplification products were cleaned with agarose gels and extracted using the NucleoSpin®-Extract II kit (MachereyNagel). Purified DNA fragments were sent to a commercial sequencing facility (Macrogen, Korea) or sequenced on an in-house Beckman-Coulter CEQ 8000 or ABI Prism 377 sequencer. The sequences were manually edited and aligned using PhyDE® (Müller *et al.,* 2005). Besides substitutions, additional phylogenetic information derived from an indel coding matrix employing the simple coding algorithm of Simmons & Ochoterena (2000), implemented in SeqState (Müller, 2005).

Reconstruction of phylogenetic hypothesis

Bayesian inference of the *trnK-psbA* region was conducted using MrBayes, version 3.0b4 (Huelsenbeck & Ronquist, 2001). The best fitting model (TIM3+G) of sequence evolution was selected using the Akaike information criterion obtained from jModeltest 0.1.1 (Posada, 2008). Six independent runs with four chains of 7 million MCMC generations each were run simultaneously saving trees every 100 generations. The first 10% of trees were discarded as burn-in after determining stationarity with Tracer v1.5 (Rambaut & Drummond, 2009). Posterior probabilities (PP) of clades were obtained from the 50% majority rule consensus tree, which was graphically displayed using TreeGraph 2 (Stöver & Müller, 2010).

Fossils and molecular dating analysis

Horn *et al.* (2003) reported leaf and pollen fossils of Piperaceae from the Pleistocene. However, in agreement with Smith *et al.* (2008) we doubt that these findings assign the origin of *Peperomia* since Piperales, the order of Piperaceae, is seen as an ancient lineage of angiosperms (ca. 150-120 MYA, e.g. Magallón 2009, 2010; Bell *et al.*, 2010). In the absence of any fossil record for direct calibration, suitable calibration points had to be found outside *Peperomia*. Fossils from Saururaceae (Mid-Eocene, set at 46 MYA, Smith & Stockey, 2007) and Lactoridaceae (91.2 MYA, Zavada & Benson, 1987; MacPhail *et al.*, 1999; Magallón & Sanderson, 2001) have been used instead. Because of their phylogenetic distance from *Tildenia* and to avoid losing variable parts of the alignment for the ingroup by adding distantly related taxa, we performed the dating analyses using a two-step approach. First, we used the published Piperales dataset of Wanke *et al.* (2007) with the above mentioned calibration points to calculate the *Piper-Peperomia* split. The resulting date was applied to the *Tildenia* dataset.

Although molecular dating methods have largely been improved, several drawbacks still exist (Sanderson *et al.* 2004). However, using some caution, they allow tests of competing biogeographical hypotheses. Two currently favoured dating methods have been applied in a comparative manner: Penalized Likelihood (PL; Sanderson, 2002) and Bayesian based "Relaxed Phylogenetics" (RP; Drummond *et al.* 2006). A relaxed molecular clock is applied in both approaches, rejecting a strict rate of evolution for a phylogenetic tree (Renner, 2005).

Relaxed phylogenetics was performed using the program BEAST v1.4.8 (Drummond & Rambaut, 2007). This program allows the integration of different molecular clock models into MCMC reconstructions. The XML command files were generated with BEAUti v.1.4.8. (Drummond & Rambaut, 2007). The best model of sequence evolution used for Bayesian analyses (TIM3+G) is not implemented in BEAST v1.4.8. Thus we used the second best

model (GTR+ G) from jModeltest 0.1.1 (Posada, 2008). “Uncorrelated Lognormal” was set as the relaxed clock model as recommended by Drummond *et al.* (2006). As tree priors, the Birth-and-Death process and lognormal distribution for fossil calibrations were chosen (Ho, 2007). The Markov chain was run with 60 million generations and trees were sampled every 1000th generation. A user-defined starting tree was arbitrarily selected from the stationary phase of the Maximum Likelihood analyses of Wanke *et al.* (2007) and used for the age estimates for the Piperales dataset. For the *Tildenia* dataset a randomly selected tree from the stationary phase was used from the MrBayes analysis performed here. Ultrametric starting trees required by BEAST were obtained by applying the program pathd8 (Britton *et al.*, 2007). Statistical convergence was verified using Tracer v1.5 (Rambaut & Drummond, 2009). The mean node heights were calculated and plotted on the maximum clade credibility tree after exclusion (burn-in) of the first 1% of trees with TreeAnnotator v1.5.4. Relaxed phylogenetic analysis of the *Tildenia* dataset gave no stable convergence. To reach statistical support, a reduced dataset including the same species sampling with one accession each was run with equal parameters without a starting tree. The results were graphically illustrated with the program FigTree v.1.2.3 (Rambaut, 2009).

For the PL analyses we used the same topology as the starting tree used for RP. Cross validation was conducted to obtain the optimal smoothing value. Increments were set at 1 with 8 steps. Estimates of node ages were determined by setting the smoothing at the optimal value of 1 obtained from cross-validation.

Distributional data and maps

To illustrate spatial patterns, a distribution map for each *Tildenia* clade was created. Distribution information derived from GPS data of more than 300 of our own collections and from georeferenced location data of approximately 1,000 herbarium specimens from 57 American and European herbaria. All data were assembled in one dataset (dbase file) and coordinates were unified in decimal degrees format. The respective file was imported into ArcView 3.3 and converted into a point shape-file, maintaining the complete record information. The layer coordinate system was defined as WGS 84 and the record shape file was complemented with other information (e.g. country borders (shape-file), and shaded relief (raster-dataset)). For each clade a map was designed using ArcGIS 9.2, except clade V, which is displayed together with clade VI.

Ancestral area reconstruction

Reconstruction of the ancestral distribution area was performed using 1) Dispersal-Vicariance Analysis (DIVA) and 2) Dispersal-Extinction-Cladogenesis (DEC). The widely used Dispersal-Vicariance Analysis implemented in the software DIVA Version 1.1 (Ronquist 1996, 1997) allows reconstructing the ancestral distribution without any presetting of general area relationships, by minimizing the number of dispersal and extinction events. DIVA requires a bifurcated tree, which is usually unavailable for most phylogenies. Therefore, we used the bifurcated maximum clade credibility phylogeny of the reduced *Tildenia* dataset created by BEAST (analysis 5). Furthermore, DIVA requires a specification of distribution area of all terminal taxon. To restrict the number of areas, the outgroup was reduced to closely related Neotropical *Peperomia* species. The entire distribution area was subdivided into seven areas: (A) Mexico, (B) Central America, (C) Venezuela/Colombia/Ecuador, (D) north and central Peru, (E) south Peru, (F) Lake Titicaca area including the Peruvian department of Puno and the Bolivian department of La Paz and (G) south Bolivia/ north Argentina.

The same distribution matrix and phylogeny was used for DEC implemented in the program LAGRANGE (Ree *et al.*, 2005; Ree & Smith, 2008). This maximum likelihood based method that calculates the most likely ancestral areas by modelling range transitions as a function of time is currently regarded as the most promising alternative to DIVA. Dispersal-extinction-cladogenesis is more complex and flexible than DIVA and incorporates a time-calibrated phylogeny (Kodandaramaiah, 2010). For the DIVA and DEC analyses, we constrained the number of maximum ancestral areas to two per node. The applied topology and the area coding matrix are provided in appendix S3.

Results

Dataset, phylogeny and current distribution of *Tildenia*

The alignment comprised 3502 characters and 316 indel coding positions. The first 12 bp of the *trn*K 5′ intron and 29 bp of the *trnK-psbA* spacer were excluded due to partially incomplete sequences. Eighteen mostly small regions of uncertain sequence homology were excluded from the data matrix (see Appendix S4). The alignments are available from TreeBASE.

The complete phylogeny, providing relationships of individual species, is provided in Appendix S5. However, only major results at the clade level are presented here because shifts in the geographic distribution of *Tildenia* are almost exclusively at the level of major clades (see Fig. 1, Fig. 2). *Tildenia* receives maximum support and is subdivided into seven mostly well-supported monophyletic clades (hereafter called clades I to VII). The South American clades I and II are successive sister lineages to the remaining clades which are all well-supported albeit unresolved with respect to each other. Four of them (clades III-VI) consist exclusively of South American species with the exception of *P. cuchumatanica.* This endemic species from Guatemala is located within clade IV (Appendix S5). All Andean clades show roughly the same distribution area, proving multiple parallel colonisations of the Andean Cordillera (Fig. 2). Clade VII contains Central American and Mexican species with the exception of the first branch (*P. chutanka),* an endemic species from the Andes near Lima (Appendix S5). Clade V was not recovered as monophyletic in the BEAST analysis (Fig. 1), but was monophyletic in the full Bayesian analyses (Fig. 2, Appendix S5). However, since clades III-VI are all Andean in their distribution, the monophyly of clade V does not alter our overall conclusions.

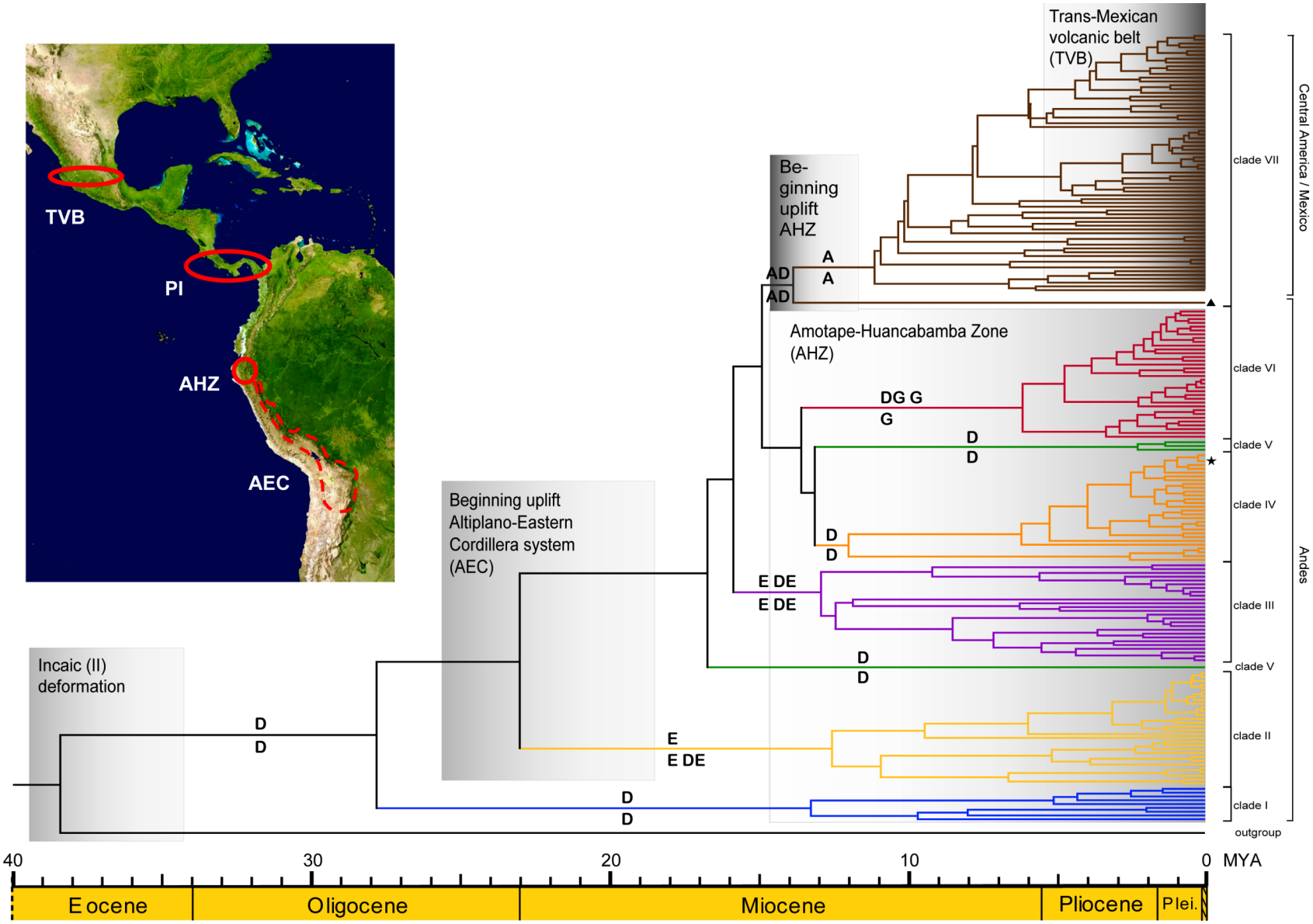

TVB
PI
AHZ
AEC
Trans-Mexican volcanic belt (TVB)
Be-ginning uplift AHZ
Amotape-Huancabamba Zone (AHZ)
Beginning uplift Altiplano-Eastern Cordillera system (AEC)
Incaic (II) deformation
clade VII
clade VI
clade V
clade IV
clade III
clade V
clade II
clade I
outgroup
Central America / Mexico
Andes
40
30
20
10
0 MYA
Eocene
Oligocene
Miocene
Pliocene
Plei.

Figure 1 (previous page). **Divergence time estimation of *Peperomia* subgenus *Tildenia* using BEAST. The maximum credibility tree is inferred from the *trnK-psbA* region. The scale bar is defined for geological periods as million years ago (MYA) (Plei.= Pleistocene, Holocene dashed). Major geological events in the respective geological era influencing the evolution of *Tildenia* are illustrated as grey zones (TVB = Trans-Mexican Volcanic Belt, AHZ = Amotape-Huancabamba Zone, AEC = Altiplano–Eastern Cordillera system) and in red on the map to the left (Incaic deformation not shown in map; PI = Panama Isthmus, see text for details). Estimated ancestral areas for main clades, constrained at maximum two, are given above (DEC) and below (DIVA) branches. Distribution areas are defined as: A) Mexico, B) Central America, C) Colombia/Venezuela/Ecuador, D) north/central Peru, E) south Peru, F) Lake Titicaca area including the Peruvian department of Puno and the Bolivian department of La Paz and G) south Bolivia /north Argentina. Clade I-VI consists of South American species and the Central American *P. cuchumatanica* (star, clade IV). Clade VII consists of the Mexican species and the South American *P. chutanka* (triangle). Clade V is not monophyletic in the BEAST analysis, but see Bayesian inference (Appendix S5).**

a
b
c
d
e
f
N
0
2.000 km
450
900 km
45°S
30°S
15°S
0°
15°N
30°N
120°W
100°W
80°W
60°W
40°W
25°S
20°S
15°S
10°S
5°S
80°W
70°W
60°W
P. bracteata (clade VII)
P. verruculosa (clade VI)
P. ocrosensis (clade V)
P. andina (clade IV)
P. cerrateae (clade III)
P. peruviana (clade II)
P. rupiseda (clade I)
clade VII
clade VI
clade V
clade IV
clade III
clade II
clade I
1
0.81
0.99

Figure 2 (previous page). **Phylogeny of *Peperomia* subgenus *Tildenia* based on 201 accessions representing 49 species forming seven clades (left) shown as a 50% majority rule Bayesian tree derived from the *trnK-psbA* region. Names have been omitted and posterior probabilities (PP) are given for major clades only. The black star indicates the position of *P. cuchumatanica* and the black triangle the position of *P. chutanka* in the phylogeny. For species names and complete support information see Appendix S5. Line drawings illustrate representative members of the different clades. a-f) distribution maps of respective clades based on ca. 1300 georeferenced accessions (triangles). The distribution areas are results of a visual interpretation of the spatial distribution patterns observed in the record dataset: a) overview map with the northern centre of distribution (clade VII, brown) and *P. cuchumatanica* (clade IV, orange triangle), b) distribution of clade V (green) and VI (red), c) clade IV (orange). d) clade III (lilac), e) clade II (yellow) and *P. chutanka* (clade VII, brown triangle) and f) clade I (blue).**

Molecular dating, lineage-through-time and ancestral area reconstruction

Penalized Likelihood (PL) and Relaxed Phylogenetics (RP) resulted in a stem age of *Peperomia* of about 57 MYA (Appendix S6, Fig. 1). The results of the different analyses (full and reduced sampling) and methods (PL and RP) range within 4 MY (Appendix S6), which confirms the accuracy of our results independently from the methods applied. *Tildenia* arose at ca. 38 MYA, speciation within the subgenus started with the split of clade I at ca. 26 MYA and the subsequent split of clade II (ca. 21 MYA). The remaining clades of *Tildenia* arose between ca. 14 and 11 MYA. The lineage-trough-time plot (Fig. 3) shows considerable increase in diversification beginning at 15 MYA. While the increase of branching rates continues in the Mexican lineage, the South American lineages show a prominent stagnation of speciation between 10-8 MYA before a second increase of lineage numbers that starts ~7 MYA and continues into the present.

DIVA and LAGRANGE largely recovered the same most probable ancestral areas of *Tildenia* and its clades (Fig. 1). For the subgenus, ancestral area reconstruction resulted in a most probable origin in northern/central Peru. For most of the clades, both methods display nearly identical results for a most probable ancestral area in northern/central Peru for clades I, IV and V and Mexico for clade VII. The results of the remaining clades are inconsistent, exposing a second probable area of origin in south Peru for clades II and III and for clade VI in south Bolivia / north Argentina (Appendix S6, graphically displayed in Fig. 1).

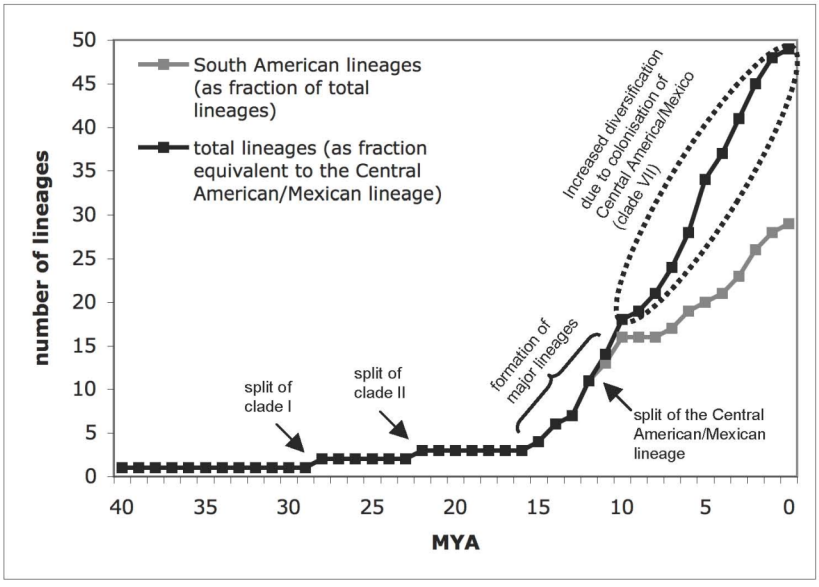


Figure 3. **Lineage-through-time plot for *Peperomia* subgenus *Tildenia*. Relative times of branching events are based on a Relaxed Phylogenetic analysis (reduced *Tildenia* dataset) of the *trnK-psbA* region. Lineages are plotted to species level only.**

Discussion

Early Andean radiation

Our study estimates the origin of *Tildenia* in north/central Peru at the end of the Eocene (Fig. 1, Table 1), supporting Wilf *et al.* (2003) who suggest high altitude diversity to be of ancient origin in South America. The spatio-temporal placement of this origin roughly coincides with the Late-Eocene deformation (Incaic (II)) of the Western Central Cordillera (e.g. Noble *et al.*, 1990; Graham, 2009). The early evolution of *Tildenia* is strongly correlated with the orogeny of the Andes. Folding of the lithosphere has always been considered a major driving force of plant speciation (e.g. Barthlott *et al*., 2005; Kreft & Jetz, 2007). The Andes especially stand out with heterogenic environmental conditions due to fissured topography (e.g. Hughes & Eastwood, 2006) and variation in temperature, precipitation and radiation exposure along this gradient (Gregory-Wodzicki, 2000). A comparable impact on the evolution of *Tildenia* also is fluctuation in temperature and precipitation in the Oligocene and Miocene (e.g. Zachos *et al.*, 2001; Hartley, 2003), leading to recurrent seasonality (Burnham & Graham, 1999). Such seasonality may have been a factor that favoured the spread, and thus current distribution of *Tildenia*. These climatic oscillations caused recurrent altitudinal shifts of vegetation zones that allowed migration between temporarily connected habitats and allopatric speciation in times of disconnection (e.g. van der Hammen, 1974; Hooghiemstra & Van der Hammen, 2004).

The first emergence of an extant *Tildenia* lineage (clade I) occurred at ca. 28 MYA in the middle Oligocene. Tectonic activity in the early Miocene led to further uplifts of the Western Cordillera and compression of the Altiplano–Eastern Cordillera (AEC) (Somoza, 1998, Gregory-Wodzicki, 2000) that co-occurred with the divergence of clade II (Fig. 1). The appearance of new habitats south-east of the ancestral area (Appendix S6, Fig.1, Fig. 2) permitted further colonisation and subsequent diversification of *Tildenia*. In this regard, high Andean regions are comparable with newly formed islands, in which new habitats and the absence of competition allow for establishment of new populations (von Hagen & Kadereit, 2001; Hughes & Eastwood, 2006).

The time between 16 and 10 MYA was a phase of large evolutionary importance for *Tildenia* as seen by the emergence of five new clades (clade III-VII, Fig. 1) and simultaneous diversification within them (Fig. 3). The increased speciation coincides with the major Andean uplift that started in the Mid-Miocene (Gregory-Wodzicki, 2000; Garzione *et al.*, 2008). Furthermore frequent environmental changes during the entire Neogene (van der Hammen & Hooghiemstra, 2000) and a major drop in temperature contributing to the spread of seasonality is reported after the Mid-Miocene Climatic Optimum (Miller *et al.*, 1987; Zachos, 2001). The increased seasonality and climate

fluctuations may have favoured speciation in *Tildenia* as populations became isolated from each other in climatically similar, but geographically separated areas.

Climate fluctuations might in particular be active in the Amotape-Huancabamba Zone (AHZ), which overlaps with the northern part of the Central Andean distribution area. The AHZ, located in the borderland of Ecuador and Peru at a latitude between ~ 2° and 8°S (Weigend, 2002; 2004), is a transition zone between the Northern and the Central Andes and a region of particular biological diversity (e.g. Weigend 2002; Richter *et al.*, 2009). As with numerous other plant groups (e.g. Stern *et al.,* 2008; Weigend *et al.*, 2010) the mosaic nature of habitats and the dynamic history of that region may have led to an increase in speciation in *Tildenia.* The impact clearly appears in clade I and IV, in which more than half of the species occur in the AHZ (Fig. 2).

Spreading from the Central Andes

The further expansion of the distribution of *Tildenia* was hindered by the lowland Amazon basin, the arid Atacama Desert, and the Pacific. The northern limit of distribution within the South American centre of diversity lies in the AHZ. The region is not only a centre of high biodiversity but also an important colonisation barrier for high altitude flora and fauna (e.g. Stern *et al.*, 2008; Cosacov *et al.*, 2009) due to its considerably lower elevations. Up to the Mid-Miocene, the AHZ was a lowland corridor with probable marine incursions (e.g. Hoorn, 1995; Lovejoy *et al.* 2006; Santos *et al.*, 2008). Due to the uplift of the Eastern Cordillera after the Mid-Miocene the AHZ was gradually uplifted (Hungerbühler *et al.*, 2002), forming a migration bridge for high altitude taxa between the Northern and Central Andes. The timing of the geomophological uplift corresponds with the split of the Central American/Mexican lineage about 12 MYA (clade VIII, Fig, 1, Fig. 2), indirectly implying a northwards colonisation. Further colonisation towards Central America via Ecuador and Colombia is still uncertain. Possible “stepping stones” for the colonisation could have been the south of Ecuador, which exhibited seasonal dry climate in the Mid-Miocene (Burnham & Graham, 1999) and the Colombian Inter-Andean Valleys with seasonality of precipitation (Pennington *et al.*, 2000). Hence, the most probable explanation based on our data is a northward-oriented colonisation after the uplift of the AHZ along the Cordillera system. Long distance dispersal (LDD) which is often used as explanation for bipolarly distributed taxa (e.g. Simpson *et al.*, 2005; Moore *et al.*, 2006) can not completely be excluded. In contrast to most other *Peperomia* lineages *Tildenia* does not have functional adaptations for LDD such as sticky fruits (Samain *et al.,* 2009; Mathieu *et al.* in press; Samain *et al.*, in press). This is reflected in our analyses where clades are largely restricted to relatively narrow distribution areas (Fig. 2), implying that widespread movement of *Tildenia* is rare. Nevertheless, LDD is the most likely explanation for the

placement of the Guatemalan species *P. cuchumatanica* in the otherwise Andean clade IV (Fig. 1, Appendix S5), which could be explained by potential phytogeographical affinities between the paramo of the Sierra de los Cuchumatanes and the high Andes. We currently do not have any explanation for the sister position of the Peruvian *P. chutanka* to the Mexican clade (clade VII, Fig. 1, Appendix S5).

Scenarios regarding the colonisation of Central America

As a consequence of the occurrence of *Tildenia* lineages on both the northern and southern American continents, competing hypotheses can be tested with respect to the mode and time of colonisation. Around 11 MYA, the first Central American/Mexican *Tildenia* lineages evolved (Appendix S6, Fig 1). Hence, the "island hopping" hypothesis, assuming suitable elevations such as the "proto-Caribbean archipelago" reported with uncertain age estimates between the Cretaceous and Eocene, the "proto-Greater Antilles" at around 50 MYA, or the "GAARlandia complex" at ca. 35 MYA can all be excluded due to our dates for diversification in *Tildenia*.

Studies agree that the closure of the Panama Isthmus was not complete before 3.5 MYA but formation started as early as ca. 12 MYA (Coates *et al.*, 2003). Other authors (Duque-Caro, 1990; Bermingham & Martin, 1998; Iturralde-Vinent & MacPhee, 1999; Krzywinski *et al.*, 2001**;** Sanmartín & Ronquist, 2004) suggested a pre-Pliocene land connection, probably occurring with sea level changes (Haq *et al.*, 1987; Westerhold *et al.*, 2005). Furthermore, the presence of an island arc is hypothesized at the current position of Panama about 15 MYA (Krzywinski *et al.*, 2001; Sanmartín & Ronquist, 2004). Based on our results (Fig. 1) and the approximate timing of geomorphological events, we can conclude that the complete closure of the Panama Isthmus falls outside our dates, but both the beginning of the closure as well as an island arc might have been stepping stones towards the northern American continent. Mirroring our results is a recent review compiling information from numerous plant and animal taxa based on molecular dates (Cody *et al.* 2010). The authors conclude that the closure of the Panama Isthmus is less important for the "Great American Biotic Interchange" than originally thought.

Throughout the Cenozoic, Central America was largely characterized by tropical forests (Burnham & Graham, 1999**)**. In the course of a long-term cooling phase after the Mid-Miocene the climate of Central America might have prohibited tropical floristic elements and favoured seasonally adapted plants such as *Tildenia.*

Recent radiations in *Tildenia*

Speciation and colonisation of plant taxa within America are often explained by multiple radiations out of ice age refugia during the Pleistocene (e.g. Toledo, 1976; Wendt, 1987; Vovides *et al.*, 2008). Originating in the 1960s the so-called refuge theory proposed by Haffer (1969) is widely used to explain speciation of different taxa within the Neotropics. However, more recent studies postulated that major parts of extant rainforest biota (Bush, 1994; Moritz *et al.*, 2000, Glor *et al.* 2001) and seasonal dry forests (Pennington *et al.*, 2004) predate the Pleistocene and are thus not restricted to Quaternary climate fluctuations only. Based on recent findings, Haffer himself (2008) extended his refuge theory to the Tertiary. Our results are in favour of this modification as almost 90% of *Tildenia* species evolved before the Pleistocene, however in a reverse sense with respect to habitat expansion of tropical lineages. Current seasonal habitat areas are probably at a minimum now and thus current *Tildenia* distribution can be seen as refuge islands. An environment that is heterogeneous in surface relief, as is present where *Tildenia* is found in both diversity centres, increases the number of possible refuges and thus promotes allopatric speciation (Rahbek & Graves, 2001).

The Mesoamerican centre of diversity is characterized by a very complex geologic history (Marshall & Liebherr, 2000) with most of the mountain uplift occurring before the radiation of *Tildenia* in Mexico. However recent volcanic activity producing fertile soils and additional high mountain habitats is reported from the Pliocene and Pleistocene (Ferrusquia-Villafranca, 1993) and has likely promoted speciation in *Tildenia*. From the TVB, the species spread into the Central Plateau and the Sierra Madre Occidental in the north, into the volcanic outcrops of the Gulf Coast (e.g. Los Tuxtlas area) in the east, into the Sierra Madre del Sur and the Sierra Madre de Chiapas in the south and southeast, and from there into the Guatemalan highlands.

Conclusion

Our analyses detected the spatio-temporal origin of *Tildenia* in north/central Peru at the end of the Eocene. Hence, colonisation direction between the two diversity centres can clearly be indicated. The south-north colonisation of the mainly high mountain species clearly is opposed to the general trend of other high altitude taxa showing mostly a north-south directed colonisation. Age estimations in our study are in favour of an island hopping scenario around 10 MYA on land patches prior to the final closure of the Panama Isthmus. Recent molecular dating results on other taxa also support an earlier “Great American Biotic Interchange” and thus are in agreement with our findings. Recurrent seasonality as a result of climate fluctuation in combination with orogeny of mountain chains and formation of volcanic belts, promoted *Tildenia* diversification and colonisation.

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Appendix

Appendix S1: List of species used in this study. Voucher information and field or garden origin including coordinates and altitude of the collection site are given. GenBank accession (will be provided upon acceptance of the manuscript) and clade numbers according to Appendix S5 are indicated.

species	lab number	voucher	origin	geographical coordinates	altitude	GenBank accession	clade
P. albonervosa G.Mathieu	Pe22	Samain *et al.* 2007-039 (GENT)	Mexico, Jalisco	19°10'23.3"N 103°28'50.9W	660 m		VII
	Pe26	Samain *et al.* 2007-046 (GENT)	Mexico, Michoacán	18°35'05.3"N 103°30'55.4"W	180 m		
P. ampla (Trel.) G.Mathieu	Pe206	Lyonnet 667 (MEXU)	Mexico, Morelos				VII
P. andina Pino	GP919	Pino 919 (USM)	Peru, Cajamarca	7°10'26"S 78°31'51"W	3030 m		IV
	Pe359	Mathieu & Symmank 2009-008	Peru, Ancash	09°06'09,9"S 77°50'18,7"W	3174 m		
	Pe393	Mathieu *et al.* 2009-119 (GENT)	Peru, Cajamarca	07°11'22,3"S 78°34'25,8"W	3616 m		
	Pe492	Mathieu & Symmank 2009-003	Peru, Ancash	09°31'37,2"S 77°32'08,4"W	3050 m		
	Pe500	Mathieu & Symmank 2009-036	Peru, La Libertad	07°47'17,6"S 78°06'45,0"W	2700 m		
P. ayacuchoana Pino & Samain	GP1114	Pino 1114 (priv. coll.)	Peru, Ayacucho				III
	Pe428	Samain *et al.* 2009-040 (GENT)	Peru, Ayacucho	12°56'37,0"S 74°01'15,0"W	3190 m		
	Pe429	Samain *et al.* 2009-042 (GENT)	Peru, Ayacucho	12°55'50,7S 74°00'18,8"W	3290 m		
	Pe430	Samain *et al.* 2009-044 (GENT)	Peru, Ayacucho	12°57'04,9"S 74°01'21,2"W	3410 m		
P. basiradicans G.Mathieu	Pe4	Samain *et al.* 2007-009 (GENT)	Mexico, Mexico	19°05'16"N 98°40'45.2"W	2170 m		VII
	Pe11	Samain *et al.* 2007-036 (GENT)	Mexico, Mexico	19°07'29.2"N 99°45'38.6"W	3920 m		
P. bracteata Hill	Pe17	Samain *et al.* 2007-013a (GENT)	Mexico, Mexico	19°30'04.5"N 98°47'51.4"W	2480 m		VII
	Pe18	Samain *et al.* 2007-011 (GENT)	Mexico, Mexico	19°37'07"N 98°49'36"W	2520 m		
	Pe19	Samain *et al.* 2007-010 (GENT)	Mexico, Mexico	19°40'31.6"N 98°40'32.7"	2690 m		
	Pe28	Samain *et al.* 2007-052 (GENT)	Mexico, Michoacán	18°55'07.4"N 99°37'38.6"W	1960 m		
	Pe29	Samain *et al.* 2007-054 (GENT)	Mexico, Michoacán	19°22'47.5"N 102°14'12.8"W	2130 m		
	Pe31	Samain *et al.* 2007-056 (GENT)	Mexico, Michoacán	19°29'57.6"N 101°49'47.4"W	2130 m		
	Pe32	Samain *et al.* 2007-057 (GENT)	Mexico, Michoacán	19°27'49.6"N 101°36'39.1"W	2260 m		
	Pe33	Samain *et al.* 2007-058 (GENT)	Mexico, Michoacán	19°32'21"N 101°42'00.5"W	1990 m		
	Pe46	Samain *et al.* 2007-112 (GENT)	Mexico, Veracruz	18°50'47.4"N 97°06'35.6"W	1250 m		
	Pe247	Breedlove 44991 (MEXU)	Mexico, Sinaloa	-	1570 m		
	Pe249	Van Devender 96-591 (MEXU)	Mexico, Sonora	28°26'5"N 108°32'53W	1460 m		
	Pe256	Hernández M. 7813 (MEXU)	Mexico, Durango	-	2300 m		
	Pe346	Véliz *et al.* 20432 (BIGU)	Guatemala, Huehuetenango	-	-		
	Pe523	Samain *et al.* 2007-085 (GENT)	Mexico, Chiapas	16°43'13.9"N 92°35'00.9"W	2160 m		
	Pe525	Samain *et al.* 2009-274 (GENT)	Mexico, Durango	24°05'56.5"N 105°40'17.9"W	2327 m		
	Pe527	Samain *et al.* 2009-272 (GENT)	Mexico, Durango	24°28'37.7"N 105°47'28.2"W	2237 m		
	Pe528	Samain *et al.* 2009-270 (GENT)	Mexico, Durango	23°41'57.0"N 105°43'16.3"W	2736 m		
	Pe529	Samain *et al.* 2009-264 (GENT)	Mexico, Durango	25°11'16.8"N 106°30'17.1"W	2450 m		
	Pe530	Samain *et al.* 2009-263 (GENT)	Mexico, Durango	25°08'33,6"N 106°25'09.7"W	2560 m		
	Pe531	Samain *et al.* 2009-262 (GENT)	Mexico, Durango	23°42'48.4"N 105°40'15.5"W	2644 m		
	Pe532	Samain *et al.* 2009-259 (GENT)	Mexico, Durango	23°41'57,0"N 105°43'16,3"W	2736 m		
	Pe533	Samain *et al.* 2009-254 (GENT)	Mexico, Durango	23°39'38.8"N 105°26'10"W	2685 m		

	Pe534	Samain *et al.* 2009-253 (GENT)	Mexico, Durango	23°39'38.8"N 105°26'10"W	2685 m		
P. bracteata Hill aff.	Pe255	García-Mendoza *et al.* 8526 (MEXU)	Mexico, Oaxaca	16°38'12"N 96°55'48.6"W	1634 m		VII
P. cavispicata G.Mathieu	Pe3	Samain *et al.* 2007-017 (GENT)	Mexico, Guerrero	18°33'46.4"N 99°37'17.4"W	1000 m		VII
	Pe14	Samain *et al.* 2007-026 (GENT)	Mexico, Mexico	18°55'07.4"N 99°37'38.6"W	1960 m		
P. cerrateae Pino & G.Mathieu	Pe111	Samain *et al.* 2008-081 (GENT)	Bolivia, Tarija	21°06'50,2"S 64°41'42,1" W	3000 m		III
	Pe171	Symmank *et al.* 2008-041 (GENT)	Bolivia, La Paz	15°43'40.9"S 68°39'26,2"W	3200 m		
	Pe222	Samain *et al.* 2008-146 (GENT)	Bolivia, Cochabamba	17°15'47,8"S 66°20'59,3"W	4080 m		
	Pe353	Mathieu *et al.* 2009-021 (GENT)	Peru, Ancash	10°09'33,1"S 77°21'06,3"W	3560 m		
	Pe371	Mathieu & Symmank 2009-041	Peru, Ancash	10°20'31.2"S 77°24'20,0"W	2359 m		
	Pe408	Samain *et al.* 2009-002 (GENT)	Peru, Lima	11°26'51"S 76°36'57"W	2750 m		
	Pe417	Samain *et al.* 2009-023 (GENT)	Peru, Junín	12°16'55,4"S 75°42'06,4"W	4010 m		
	Pe433	Samain *et al.* 2009-055 (GENT)	Peru, Ayacucho	13°23'18,5"S 73°57'38,1"W	4090 m		
	Pe434	Samain *et al.* 2009-057 (GENT)	Peru, Ayacucho	13°23'57,4"S 73°56'52,3"W	4020 m		
P. chutanka Pino	Pe436	Samain *et al.* 2009-076 (GENT)	Peru, Lima	12°09'17"S 76°23'40"W	3320 m		VII
P. claytonioides Kunth	Pe202	Linares 607 (MEXU)	El Salvador, Sonsonate	-	-		VII
P. cuchumatanica Véliz	Pe348	Véliz & Vélasquez 20297 (BIGU)	Guatemala, Huehuetenango	-	-		IV
P. cyclaminoides Hill	Pe110	Samain *et al.* 2008-080 (GENT)	Bolivia, Tarija	21°09'16,5"S 64°41'32,0"W	2940 m		VI
	Pe114	Samain *et al.* 2008-084 (GENT)	Bolivia, Tarija	21°27'20,8"S 64°52'39,6"W	3130 m		
	Pe115	Samain *et al.* 2008-085 (GENT)	Bolivia, Tarija	21°29'52,7"S 64°54'11,3"W	3825 m		
	Pe117	Samain *et al.* 2008-087 (GENT)	Bolivia, Chuquisaca	20°45'00,1"S 64°52'20,2"W	3130 m		
	Pe118	Samain *et al.* 2008-088 (GENT)	Bolivia, Chuquisaca	20°44'29,1"S 64°48'31,7"W	3220 m		
	Pe218	Samain *et al.* 2008-129 (GENT)	Bolivia, Cochabamba	17°46'09,9"S 65°29'32,8"W	3420 m		
	Pe219	Samain *et al.* 2008-130 (GENT)	Bolivia, Cochabamba	17°45'0,6"S 65°29'57,4"W	3580 m		
	Pe220	Samain *et al.* 2008-131 (GENT)	Bolivia, Cochabamba	17°44'11,6"S 65°31'00,9"W	3685 m		
P. dolabella Rauh & Kimnach	P152	Pino 200 (USM)	Peru, Cajamarca	7°12'15"S 78°30'24"W	2900 m		IV
	RM254	Pino 885 (USM)	Peru, Cajamarca	7°12'19"S 78°30'24"W	3000 m		
	Pe392	Mathieu *et al.* 2009-118 (GENT)	Peru, Cajamarca	07°10'34,1"S 78°31'53,8"W	3132 m		
	Pe396	Mathieu *et al.* 2009-125 (GENT)	Peru, Cajamarca	07°12'17,4"S 78°30'25,9"W	2992 m		
P. elatior G.Mathieu	Pe12	Samain *et al.* 2007-035 (GENT)	Mexico, Mexico	18°52'33.4"N 100°17'09"W	1500 m		VII
P. exclamationis G.Mathieu	Pe25	Samain *et al.* 2007-045 (GENT)	Mexico, Michoacán	18°41'20.1"N 103°24'44.6"W	1260 m		VII
P. gigantea G.Mathieu	Pe358	Mathieu *et al.* 2009-158 (GENT)	Peru, Cajamarca	07°09'25,5"S 78°50'30,4"W	1770 m		I
P. gracillima Wats. aff.	Pe16	Samain *et al.* 2007-013b (GENT)	Mexico, Mexico	17°17'10"N 99°28'20"W	750 m		VII
	Pe23	Samain *et al.* 2007-042 (GENT)	Mexico, Jalisco	20°07'40.2"N 103°11'19.4"W	1575 m		
P. jalcaensis Pino	Pe394	Mathieu *et al.* 2009-120 (GENT)	Peru, Cajamarca	07°11'19,8"S 78°34'32,3"W	3639 m		IV
P. klopfensteinii Pino & Cieza	Pe356	Mathieu *et al.* 2009-135 (GENT)	Peru, Cajamarca	06°13'44,4"S 78°45'15,0"W	1548 m		I
	Pe384	Mathieu *et al.* 2009-104 (GENT)	Peru, Amazonas	06°08'21,0"S 77°59'26,1"W	2797 m		
	Pe397	Mathieu *et al.* 2009-130 (GENT)	Peru, Cajamarca	06°39'32,3"S 78°44'01,4"W	2450 m		
P. lilliputiana (Pino & Cieza) Pino & Cieza	Pe361	Mathieu & Symmank 2009-024	Peru, La Libertad	07°53'03,1"S 78°07'21,2"W	3350 m		IV
	Pe370	Mathieu & Symmank 2009-038	Peru, La Libertad	07°53'15,2"S 78°07'13,5"W	3565 m		
P. macrandra C.DC.	Pe13	Samain *et al.* 2007-032 (GENT)	Mexico, Mexico	18°52'19"N 99°38'18"W	1720 m		VII
	Pe50	Samain *et al.* 2007-099 (GENT)	Mexico, Oaxaca	17°35'08.1"N 96°30'41"W	2650 m		
	Pe201	Croat & Hannon 65637 (MEXU)	Mexico, Oaxaca	17°37'00"N 96°23'00"W	2580 m		

	Pe203	Martínez 19592 (MEXU)	Guatemala, San Marcos	-	2750 m		VII
	Pe205	Miller & Campos 2904 (MO)	Mexico, Guerrero	17°20'00"N 100°04'00"W	2400 m		
	Pe260	Martínez 13126 (MEXU)	Guatemala, Baja Verapaz	-	1660 m		
P. macrorhiza Kunth	RM275	Pino 327 (USM)	Peru, Cajamarca	7°11'24"S 78°21'27"W	2800 m		IV
	Pe354	Mathieu & Symmank 2009-039	Peru, La Libertad	07°53'15,2"S 78°07'13,5"W	3565 m		
P. mexicana (Miq.) Miq.	Pe45	Samain *et al.* 2007-115 (GENT)	Mexico, Veracruz	18°34'43.4"N 95°05'59.3"W	225 m		VII
	Pe200	Martínez 33807 (MEXU)	Mexico, Veracruz	17°37'23"N 94°01'51"W	80 m		
	Pe252	Campos 3499 (MEXU)	Mexico, Oaxaca	16°12'00"N 96°57'00"W	1500 m		
P. monticola Miq.	Pe257	Díaz Vilchis 1427 (MEXU)	Mexico, Hidalgo	20°09'44"N 99°01'30"W	2300 m		VII
P. moralesii Véliz	Pe347	Véliz & Vélasquez 20330 (BIGU)	Guatemala, Huehuetenango	-	-		VII
P. occulta G.Mathieu	Pe49	Samain *et al.* 2007-088 (GENT)	Mexico, Chiapas	16°51'42.9"N 92°27'13.9"W	1320 m		VII
	Pe582	Samain *et al.* 2007-098 (GENT)	Mexico, Oaxaca	17°35'15.1"N 96°29'21.8"W	2265 m		
	Pe583	Samain *et al.* 2007-095 (GENT)	Mexico, Oaxaca	17°36'07.6"N 96°22'44"W	1635 m		
P. ocrosensis G. Mathieu & Pino	Pe355	Mathieu *et al.* 2009-042 (GENT)	Peru, Ancash	10°27'32,4"S 77°24'29,6"W	3104 m		V
P. ovatopeltata C.DC.	Pe20	Samain *et al.* 2007-002 (GENT)	Mexico, Michoacán	19°30'39"N 101°50'02"W	2170 m		VII
	Pe30	Samain *et al.* 2007-055 (GENT)	Mexico, Michoacán	19°25'34.3"N 102°04'10.5"W	1600 m		
P. ovatopeltata C.DC. aff.	Pe41	Samain *et al.* 2007-124 (GENT)	Mexico, Morelos	19°01'11.3"N 99°09'12"W	2260 m		VII
	Pe42	Samain *et al.* 2007-122 (GENT)	Mexico, Morelos	18°59'10.7"N 99°06'07.7"W	2000 m		
	Pe43	Samain *et al.* 2007-120 (GENT)	Mexico, Morelos	18°59'10.7"N 99°06'07.7"W	1725 m		
	Pe44	Samain *et al.* 2007-119 (GENT)	Mexico, Morelos	18°59'10.7"N 99°06'07.7"W	1700 m		
P. parvifolia C.DC.	Pe172	Symmank *et al.* 2008-043 (GENT)	Bolivia, La Paz	15°41'53.1"S 68°37'34,1"W	4000 m		IV
	Pe357	Mathieu & Symmank 2009-011	Peru, Ancash	09°33'18,1"S 77°38'09,3"W	4125 m		
	Pe420	Samain *et al.* 2009-027 (GENT)	Peru, Huancavelica	12°20'40,8"S 74°56'13,0"W	4180 m		
	Pe427	Samain *et al.* 2009-038 (GENT)	Peru, Ayacucho	12°59'38,7"S 74°05'52,6"W	4040 m		
P. parvisagittata G.Mathieu & Pino	Pe113	Samain *et al.* 2008-083 (GENT)	Bolivia, Tarija	21°11'14,6"S 64°46'18,9"W	3060 m		III
	Pe352	Mathieu & Symmank 2009-020	Peru, Ancash	10°09'31,4"S 77°21'13,3"W	3480 m		
	Pe409	Samain *et al.* 2009-003 (GENT)	Peru, Lima	11°29'10,8"S 76°38'37,6"W	2400 m		
P. pedicellata Dahlst.	Pe36	Samain *et al.* 2007-067 (GENT)	Mexico, Chiapas	15°25'38.2"N 92°20'36.3"W	2810 m		VII
	Pe37	Samain *et al.* 2007-072 (GENT)	Mexico, Chiapas	15°28'30.9"N 92°16'44.9"W	2620 m		
P. peruviana Dahlst.	Pe80	Samain *et al.* 2008-015 (GENT)	Bolivia, Cochabamba	17°24'03,3"S 65°48'24,9"W	3180 m		II
	Pe82	Samain *et al.* 2008-013 (GENT)	Bolivia, Cochabamba	17°42'46,2"S 66°33'14,2"W	3930 m		
	Pe104	Samain *et al.* 2008-024 (GENT)	Bolivia, Cochabamba	17°27'25,6"S 65°44'10,7"W	3185 m		
	Pe108	Samain *et al.* 2008-075 (GENT)	Bolivia, Tarija	21°26'29,8"S 64°23'08,8"W	2335 m		
	Pe109	Samain *et al.* 2008-076 (GENT)	Bolivia, Tarija	21°25'04,1"S 64°25'52,8"W	2550 m		
	Pe112	Samain *et al.* 2008-082A (GENT)	Bolivia, Tarija	21°11'14,6"S 64°46'18,9"W	3060 m		
	Pe116	Samain *et al.* 2008-086 (GENT)	Bolivia, Chuquisaca	20°46'06,4"S 64°52'20,2"W	2940 m		
	Pe119	Samain et al. 2008-091 (GENT)	Bolivia, Chuquisaca	19°10'32,5"S 64°54'51,5"W	3275 m		
	Pe120	Samain *et al.* 2008-089 (GENT)	Bolivia, Potosí	19°33'11,0"S 65°27'25,0"W	3150 m		
	Pe168	Symmank *et al.* 2008-036 (GENT)	Bolivia, La Paz	15°44'14,8"S 68°41'54,0"W	2250 m		
	Pe173	Symmank *et al.* 2008-045 (GENT)	Bolivia, La Paz	15°27'51.4"S 69°05'24,1"W	3925 m		
	Pe188	Symmank & Mathieu 2008-034	Bolivia, La Paz	16°03'29.5"S 68°40'46,4"W	3700 m		

	Pe208	Samain *et al.* 2008-092 (GENT)	Bolivia, Chuquisaca	19°04'52,7"S 64°49'23,2"W	2755 m		
	Pe212	Samain *et al.* 2008-105B (GENT)	Bolivia, Chuquisaca	18°41'36,3"S 64°41'37,3"W	2670 m		
	Pe438	Samain *et al.* 2009-081 (GENT)	Peru, Puno	15°39'41,3"S 70°28'13,0"W	3950 m		
	Pe440	Samain *et al.* 2009-095 (GENT)	Peru, Puno	15°02'45,6"S 70°22'26,3"W	3940 m		
	Pe441	Samain *et al.* 2009-098 (GENT)	Peru, Puno	14°36'42,1"S 70°45'28,0"W	4010 m		
	Pe135	Pino s.n. (priv. coll.)	Peru, Arequipa	-	-		
	Pe443	Samain *et al.* 2009-105A (GENT)	Peru, Cuzco	13°34'39,1"S 71°42'41,9"W	3130 m		
	Pe445	Samain *et al.* 2009-112 (GENT)	Peru, Cuzco	13°18'23,4"S 72°02'51,8"W	3200 m		
P. polycephala Trel.	Pe446	Samain *et al.* 2009-113 (GENT)	Peru, Cuzco	13°20'33,7"S 71°56'40,3"W	2950 m		II
	Pe450	Samain *et al.* 2009-115 (GENT)	Peru, Cuzco	13°24'36,0"S 71°50'33,3"	3330 m		
	Pe451	Samain *et al.* 2009-116 (GENT)	Peru, Cuzco	13°08'30,6"S 72°35'01,6"W	1640 m		
	Pe574	Samain *et al.* 2009-045 (GENT)	Peru, Ayacucho	12°45'08,6"S 74°22'37,7"W	2920 m		
P. pseudoperuviana (Pino) Pino	Pe575	Pino 642 (USM)	Peru, Cajamarca	7°17'35"S 78°29'18"W	2400 m		IV
	P151	Pino 894 (USM)	Peru, Cajamarca	7°17'31"S 78°29'27"W	2370 m		
	Pe426	Samain *et al.* 2009-036 (GENT)	Peru, Huancavelica	13°16'52,3"S 74°18'25,7"W	3730 m		
	Pe432	Samain *et al.* 2009-052 (GENT)	Peru, Ayacucho	13°18'55,7"S 73°58'48,8"W	3450 m		
	Pe437	Samain *et al.* 2009-077 (GENT)	Peru, Arequipa	15°36'41,9"S 15°36'41,9"W	3680 m		
	Pe455	Samain *et al.* 2009-122 (GENT)	Peru, Cuzco	13°31'05,5"S 71°52'14,0"W	3890 m		
P. pseudoverruculosa G.Mathieu	Pe456	Samain *et al.* 2009-123 (GENT)	Peru, Cuzco	13°30'08,3"S 71°58'48,2"W	3660 m		III
	Pe457	Samain *et al.* 2009-124 (GENT)	Peru, Cuzco	13°29'47,8"S 71°58'24,1"W	3740 m		
	Pe567	Samain *et al.* 2009-061 (GENT)	Peru, Apurimac	13°32'22,3"S 73°40'24,2"	3290 m		
	Pe568	Samain *et al.* 2009-062 (GENT)	Peru, Apurimac	13°32'48,4"S 73°37'43,2"W	3760 m		
	Pe571	Samain *et al.* 2009-072 (GENT)	Peru, Apurimac	13°42'01,3"S 73°02'47,4"W	3740 m		
P. pugnicaudex Pino	Pe412	Samain *et al.* 2009-007 (GENT)	Peru, Lima	11°44"24,4"S 76°16'48,7"W	3430 m		V
P. purpureonervosa G.Mathieu	Pe174	Symmank *et al.* 2008-051 (GENT)	Bolivia, La Paz	16°18'54.8"S 67°55'28,4"W	3270 m		II
P. putlaensis G.Mathieu	Pe9	Samain *et al.* 2007-105 (GENT)	Mexico, Oaxaca	17°07'51.1"N 97°52'20.2"W	1315 m		VII
	Pe48	Samain *et al.* 2007-104 (GENT)	Mexico, Oaxaca	17°11'57.6"N 97°53'17.2"W	2015 m		
*P. querococha*na G.Mathieu	Pe372	Mathieu *et al.* 2009-043 (GENT)	Peru, Ancash	10°20'08,1"S 77°20'88,0"W	3956 m		V
	Pe566	Mathieu & Symmank 2009-017a	Peru, Ancash	09°43'40.2"S 77°19'40.5"W	4100 m		
P. questionis G.Mathieu	Pe15	Samain *et al.* 2007-014 (GENT)	Mexico, Guerrero	17°29'00.2"N 99°25'45.2"W	1060 m		VII
	Pe588	German *et al.* 520 (MEXU)	Mexico, Guerrero	-	-		
	Pe61	Samain *et al.* 2008-028 (GENT)	Bolivia, Cochabamba	17°33'35,6"S 65°20'25,4"W	3030 m		
	Pe62	Samain *et al.* 2008-029 (GENT)	Bolivia, Cochabamba	17°44'38,2"S 64°58'33,4"W	3015 m		
	Pe81	Samain *et al.* 2008-014 (GENT)	Bolivia, Cochabamba	17°23'06,0"S 66°08'07,3"W	2830 m		
P. radiatinervosa G.Mathieu	Pe121	Samain *et al.* 2008-090 (GENT)	Bolivia, Chuquisaca	19°05'11,2"S 65°13'14,5"W	2900 m		VI
	Pe209	Samain *et al.* 2008-101 (GENT)	Bolivia, Chuquisaca	18°42'14,1"S 64°41'41,5"W	2535 m		
	Pe211	Samain *et al.* 2008-105A (GENT)	Bolivia, Chuquisaca	18°41'36,3"S 64°41'37,3"W	2670 m		
	Pe215	Samain *et al.* 2008-110 (GENT)	Bolivia, Chuquisaca	18°49'43,6"S 64°34'57,2"W	2625 m		
	Pe217	Samain *et al.* 2008-127 (GENT)	Bolivia, Cochabamba	18°11'46,3"S 65°16'14,0"W	2890 m		
	Pe350	Mathieu *et al.* 2009-005 (GENT)	Peru, Ancash	09°25'48,3"S 77°32'58,6"W	3055 m		
	Pe367	Mathieu & Symmank 2009-032	Peru, La Libertad	07°48'45,2"S 77°52'58,0"W	2997 m		

P. rupiseda C.DC.	Pe407	Samain *et al.* 2009-001 (GENT)	Peru, Lima	11°26'51''N 76°36'57''W	2750 m		I
	Pe413	Samain *et al.* 2009-008 (GENT)	Peru, Lima	11°08'52,8''S 76°21'25,6''W	2680 m		
	Pe576	Pino 1885 (USM)	Peru, Cajamarca	-	-		
P. schizandra Trel.	Pe21	Samain *et al.* 2007-038 (GENT)	Mexico, Jalisco	20°49'36.4''N 103°20'47.2''W	1250 m		VII
	Pe24	Samain *et al.* 2007-043 (GENT)	Mexico, Michoacán	18°95'32.7''N 103°03'36.3''W	780 m		
	Pe27	Samain *et al.* 2007-049 (GENT)	Mexico, Michoacán	18°24'11.1''N 102°22'27.1''W	700 m		
	Pe265	Samain *et al.* 2008-151 (GENT)	Mexico, Guerrero	18°36'24.7''N 99°31'28.3''W	1350 m		
P. schizandra Trel. aff.	Pe250	Cedillo T. 1621 (MEXU)	Mexico, Oaxaca	-	-		VII
P. scutellifolia Ruiz & Pav.	Pe579	Pino 2439 (USM)	Peru, Arequipa	15°46'56''S 74°23'13''W	1016 m		III
P. sp.	Pe2	Samain *et al.* 2007-023 (GENT)	Mexico, Guerrero	18°37'12.3''N 99°38'14.9''W	2330 m		VII
P. sp.	Pe421	Samain *et al.* 2009-028 (GENT)	Peru, Huancavelica	12°22'02,4S 74°48'20,7''W	3560 m		II
P. sp.	Pe422	Samain *et al.* 2009-029 (GENT)	Peru, Huancavelica	12°22'02,4S 74°48'20,7''W	3560 m		II
P. tepoztecoana G.Mathieu	Pe10	Samain *et al.* 2007-121 (GENT)	Mexico, Morelos	18°59'10.7''N 99°06'07.7''W	1950 m		VII
P. umbilicata Ruiz & Pav.	Pe324	Pino 1375 (USM)	Peru, Lima	11°21'17''S 77°21'56''W	590 m		IV
	Pe578	Pino 1379 (USM)	Peru, Lima	11°19'32''S 77°27'14''W	420 m		
	Pe580	Pino 1631 (USM)	Peru, Lima	-	-		
	Pe581	Pino 1406 (USM)	Peru, Lima	-	-		
P. umbrosa G.Mathieu	Pe395	Mathieu *et al.* 2009-122 (GENT)	Peru, Cajamarca	07°11'29,7''S 78°34'48,0''W	3557 m		II
	Pe479	Samain *et al.* 2009-210a (GENT)	Peru, Pasco	11°17'27,9''S 75°54'47,6''W	4190 m		
	Pe570	Samain *et al.* 2009-067 (GENT)	Peru, Apurimac	13°40'20,3''S 73°11'48,4''	4160 m		
P. verruculosa Dahlst. ex Hill	Pe105	Samain *et al.* 2008-027 (GENT)	Bolivia, Cochabamba	12°20'40,8S 74°56'13,0''W	4180 m		VI
	Pe351	Mathieu & Symmank 2009-007	Peru, Ancash	09°06'47,8''S 77°52'19,2''W	4270 m		
	Pe410	Samain *et al.* 2009-004 (GENT)	Peru, Lima	11°42'15,6''S 76°16'04,9''W	3790 m		
	Pe414	Samain *et al.* 2009-009 (GENT)	Peru, Junín	11°27'07''S 75°56'56,6''W	3810 m		
	Pe415	Samain *et al.* 2009-011 (GENT)	Peru, Junín	11°32'55,7''S 75°37'31,8''W	4130 m		
	Pe416	Samain *et al.* 2009-017 (GENT)	Peru, Junín	11°59'59,0''S 75°27'22,1''W	3480 m		
	Pe418	Samain *et al.* 2009-024 (GENT)	Peru, Junín	12°12'10,7''S 75°07'41,7''W	3700 m		
	Pe419	Samain *et al.* 2009-025 (GENT)	Peru, Huancavelica	12°16'11,9''S 75°03'34,4''W	3810 m		
	Pe424	Samain *et al.* 2009-031 (GENT)	Peru, Huancavelica	12°18'19,7''S 75°04'07,3''W	3930 m		
	Pe425	Samain *et al.* 2009-034 (GENT)	Peru, Huancavelica	12°48'49,1''S 75°03'24,0''	3850 m		
	Pe435	Samain *et al.* 2009-058 (GENT)	Peru, Ayacucho	13°23'21,5''S 73°57'16,1''W	3960 m		
	Pe442	Samain *et al.* 2009-101 (GENT)	Peru, Puno	14°36'23,0''S 14°36'23,0''W	4060 m		
	Pe453	Samain et al. 2009-120 (GENT)	Perú, Cuzco	13°22'42,0''S 72°03'31,0''W	3780 m		
	Pe454	Samain et al. 2009-121 (GENT)	Perú, Cuzco	13°22'42,0''S 72°03'31,0''W	3780 m		
	Pe478	Samain *et al.* 2009-208 (GENT)	Peru, Junín	11°16'40,4''S 75°46'23,3''W	3440 m		
	Pe569	Samain *et al.* 2009-063 (GENT)	Peru, Apurimac	13°32'48,4''S 73°37'43,2''W	3760 m		
	Pe572	Samain *et al.* 2009-020 (GENT)	Peru, Junín	12°05'13,5''S 75°32'02,2''W	3940 m		
	Pe573	Samain *et al.* 2009-051 (GENT)	Peru, Ayacucho	13°18'55,7''S 73°58'48,8''W	3450 m		
P. wernerrauhii Pino & Samain	Pe481	Samain *et al.* 2009-213 (GENT)	Peru, Huánuco	10°14'26,5''S 76°09'13,2''W	2700 m		IV
	Pe482	Samain *et al.* 2009-214 (GENT)	Peru, Huánuco	09°53'36,8''S 76°09'03,2''W	2470 m		
	Pe487	Samain *et al.* 2009-248 (GENT)	Peru, Huánuco	09°47'58,4''S 76°03'26,5''W	2380 m		

outgroup							
Gymnotheca chinensis Decne.		Wanke 004 (DR)	BG Bonn, 17072	-	-		
Houttuynia cordata Thunb.		Borsch 3481 (BONN)	BG Bonn, 08120	-	-		
Peperomia hispidula (Sw.) A.Dietr.	Pe503	Mathieu *et al.* 2009-052 (GENT)	Peru, Cajamarca	05°19'27,7"S 79°21'37,7"W	2672 m		
Peperomia hispiduliformis Trel.	Pe169	Symmank *et al.* 2008-037 (GENT)	Bolivia, La Paz	15°45'08.8"S 68°39'11,5"W	2680 m		out-group
Peperomia mandonii C.DC.	Pe170	Symmank *et al.* 2008-039 (GENT)	Bolivia, La Paz	15°45'08.8"S 68°39'11,5"W	2680 m		
Piper crocatum Ruiz & Pav.		Wanke 070 (DR)	BG Bonn, 18143	-	-		
Saururus cernuus L.		Borsch & Wilde 3108 (VPI, FR)	USA, Florida	-	-		
Saururus chinensis (Lour.) Baill.		Wanke 001 (DR)	BG Bonn, 00312	-	-		

Appendix S2: **Summary of primers used in the present study. The direction of the primers is indicated as for. = forward and rev. = reverse.**

Primer name	Direction	Sequence 5´-3´	Reference
MG15	for.	ATC TGG GTT GCT AAC TCA ATG	Liang and Hilu (1996)
Pe-matK-1200F	for.	TTCCAAAATCAAAAGAGCGATAGG	this study
Pe-matK-950F	for.	ATCAAAAGAGCGATAGGATTGC	this study
Pe-matK-2000F	for.	TTC CTT ACG AAT CCA TAG A	Wanke *et al.* (2006)
Pe-matK-2000R	rev.	TCTATGGATTCGTAAGGAA	this study
Pi-matK-1850R	rev.	CCAGGCAAGATACTAAT	this study
Pe-matK-2225F	for.	CCTACCCTATTCATCCAGAAAT	this study
Pe-matK-2500R	rev.	TTC GCA ATA AAT GCA AAG AGG	Wanke *et al.* (2006)
Pe-matK-2700F	for.	AAACAATCTTTTCATTTACG	Wanke *et al.* (2006)
Pe-matK-2190F	for.	TTCTTGAACGAATCCATTTCTATG	this study
Pi-matK-730R	rev.	ATAGAAATGGA(CT)TCGTTCAAG	Wanke *et al.* (2006)
Pe-matK-2140F	for.	ACTTTATCTATTTATGGCAATG	Samain *et al.* (in press)
Pe-matK-2370R	rev.	ATTACWTTGCCATAAATAGAKAA	this study
Pe-matK-2340R	rev.	TTTTCTTTTGACATTGAACCAA	Samain *et al.* (in press)
Pe-matK-2800F	for.	AATCTTTCTCATTATTATAGTGG	this study
MG1	rev.	AAC TAG TCG GAT GGA GTA GAT	Liang and Hilu (1996)
psbA-R	rev.	CGCGTCTCTCTAAAATTGCAGTCAT	Steele and Vilgalys (1994)

Appendix S3: **Topology of the reduced *Peperomia* subgenus *Tildenia* dataset and area codingmatrix used for ancestral area reconstruction with DIVA and Lagrange.**

Topology in Newick format including branch length:

((((((((((tepoztecoana:7.38457975983,(((pedicellata:5.62746264947,(putlaensis:4.40087529871,claytonioides:4.40087529871)N4:1.22658735076)N5:0.573347088546,(monticola:5.28650165423,basiradicans:5.28650165423)N8:0.91430808378)N9:0.560925467863,(((exclamationis:3.53597147699,(gracillima:2.7766268208,cavispicata:2.7766268208)N13:0.759344656202)N14:0.878438227346,ovatopeltata:4.41440970434)N16:0.952210541453,(((questionis:1.51707573616,bracteata:1.51707573616)N19:1.51503135306,elatior:3.03210708921)N21:1.72182931546,ampla:4.75393640468)N23:0.61268384112)N24:1.39511496008)N25:0.622844553945)N26:1.35073214215,((mexicana:4.60885654703,occulta:4.60885654703)N29:3.63473622564,moralesii:8.24359277268)N31:0.491719129307)N32:1.10612978173,(macrandra:8.09601901617,(schizandra:6.34110939596,albonervosa:6.34110939596)N36:1.75490962021)N37:1.74542266753)N38:2.0027827755,chutanka:11.8442244592)N40:0.808327694695,((cyclaminoides:3.43428693954,verruculosa:3.43428693954)N43:1.82373805961,radiatinervosa:5.25802499915)N45:7.39452715474)N46:0.727460248236,(umbilicata:11.3007659292,(jalcaensis:6.06962290647,(macrorhiza:4.90847116869,((wernerrauhii:2.39305340354,(lilliputiana:1.57750322492,(andina:1.18446514463,(cuchumatanica:0.71011309187,parvifolia:0.71011309187)N55:0.474352052762)N56:0.393038080287)N57:0.81555017862)N58:1.13267896102,(pseudoperuviana:2.53305262303,dolabella:2.53305262303)N61:0.992679741523)N62:1.38273880414)N63:1.16115173778)N64:5.23114302278)N65:2.07924647287)N66:1.33920709773,((scutellifolia:11.1008046098,(parvisagittata:10.5158824138,(cerrateae:9.94445763618,(ayacuchoana:6.12793624113,pseudoverruculosa:6.12793624113)N72:3.81652139504)N73:0.571424777679)N74:0.584922196007)N75:2.64491222852,((querocochana:1.80023782497,ocrosensis:1.80023782497)N78:10.1971479887,pugnicaudex:11.9973858136)N80:1.74833102473)N81:0.973502661494)N82:7.09878697706,((umbrosa:9.60187383661,(peruviana:7.2310586404,purpureonervosa:7.2310586404)N86:2.3708151962)N87:2.61750543294,polycephala:12.2193792695)N89:9.59862720743)N90:5.21944427227,((gigantea:9.76132030047,klopfensteinii:9.76132030047)N93:2.10018457409,rupiseda:11.8615048746)N95:15.1759458747)N96:10.9625492508,(mandonii:32.740813047,(hispiduliformis:5.78518306821,hispidula:5.78518306821)N100:26.9556299788)N101:5.25918695293)N102:0.0;

Defined areas of distribution and distribution matrix (0 absent, 1 present):

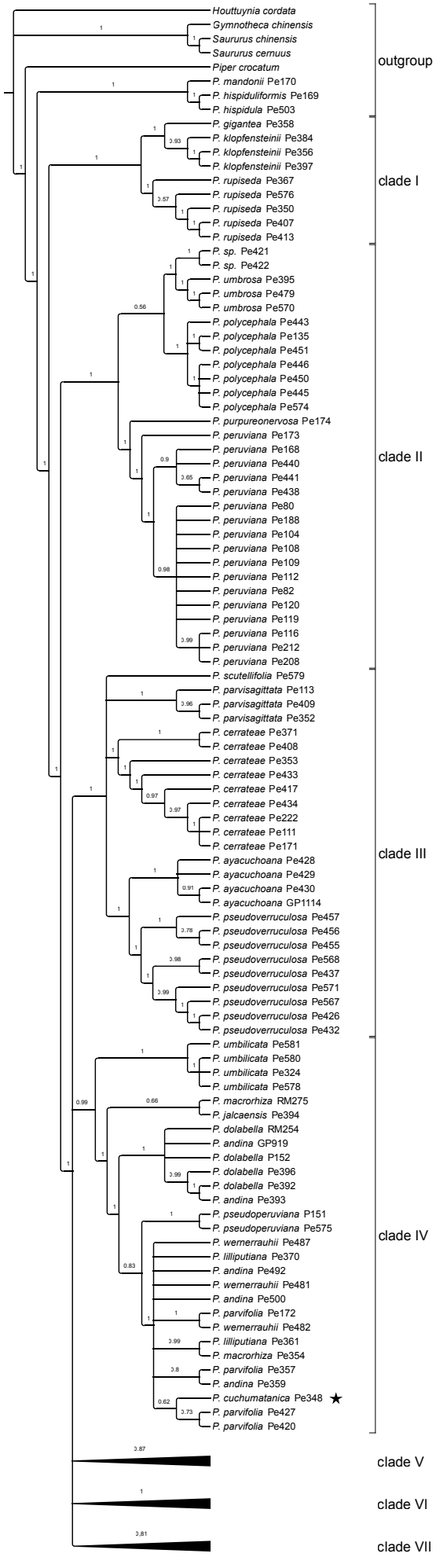
A Mexico
B Central America
C Colombia / Venezuela / Ecuador
D north/central Peru
E south Peru
F Lake Titicaca area including the Peruvian department of Puno and the Bolivian department of La Paz
G south Bolivia/ north Argentina

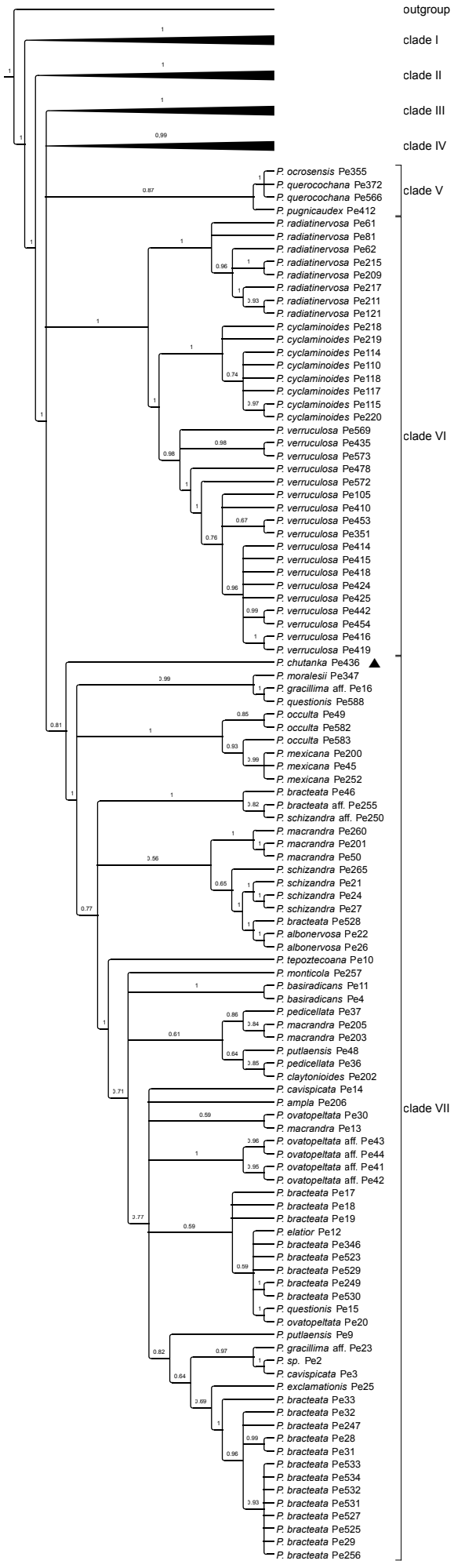
	A	B	C	D	E	F	G
albonervosa	1	0	0	0	0	0	0
ampla	1	0	0	0	0	0	0
andina	0	0	0	1	0	0	0
ayacuchoana	0	0	0	0	1	0	0
basiradicans	1	0	0	0	0	0	0
bracteata	1	1	0	0	0	0	0
cavispicata	1	0	0	0	0	0	0
cerrateae	0	0	0	1	1	1	0
chutanka	0	0	0	1	0	0	0
claytonioides	1	1	0	0	0	0	0
cuchumatanica	0	1	0	0	0	0	0
cyclaminoides	0	0	0	0	0	0	1
dolabella	0	0	0	1	0	0	0
elatior	1	0	0	0	0	0	0
exclamationis	1	0	0	0	0	0	0
gigantea	0	0	0	1	0	0	0
gracillima	1	0	0	0	0	0	0
hispidula	1	1	0	0	0	0	0
hispiduliformis	1	1	0	0	0	0	0
jalcaensis	0	0	0	1	0	0	0
klopfensteinii	0	0	0	1	0	0	0
lilliputiana	0	0	0	1	0	0	0
macrandra	1	1	0	0	0	0	0
macrorhiza	0	0	0	1	0	0	0
mandonii	0	0	0	0	0	1	1
mexicana	1	1	0	0	0	0	0
monticola	1	0	0	0	0	0	0
moralesii	0	1	0	0	0	0	0
occulta	1	0	0	0	0	0	0
ocrosensis	0	0	0	1	0	0	0
ovatopeltata	1	1	0	0	0	0	0
parvifolia	0	0	0	1	1	1	0
parvisagittata	0	0	0	1	0	0	1
pedicellata	1	1	0	0	0	0	0
peruviana	0	0	1	0	1	1	1
polycephala	0	0	0	0	1	0	0
pseudoperuviana	0	0	0	1	0	0	0
pseudoverruculosa	0	0	0	0	1	0	0
pugnicaudex	0	0	0	1	0	0	0
purpureonervosa	0	0	0	0	0	1	0
putlaensis	1	0	0	0	0	0	0
querocochana	0	0	0	1	0	0	0
questionis	1	0	0	0	0	0	0
radiatinervosa	0	0	0	0	0	0	1
rupiseda	0	0	0	1	1	0	0
schizandra	1	0	0	0	0	0	0
scutellifolia	0	0	0	0	1	0	0
tepoztecoana	1	0	0	0	0	0	0
umbilicata	0	0	0	1	0	0	0
umbrosa	0	0	0	1	1	0	0
verruculosa	0	0	0	1	1	1	1
wernerrauhii	0	0	0	1	0	0	0

Appendix S4: Regions of uncertain sequence homology (hotspots) excluded from the *trnK-psbA* alignment for the final phylogenetic analysis. Positions are given equivalent to the file downloadable from TreeBase.

	alignment position
hotspot 1	246-249
hotspot 2	392-401
hotspot 3	435-443
hotspot 4	489-499
hotspot 5	601-608
hotspot 6	768
hotspot 7	1170-1177
hotspot 8	2756-2786
hotspot 9	2965-2971
hotspot 10	3030-3038
hotspot 11	3045-3052
hotspot 12	3087-3093
hotspot 13	3261-3268
hotspot 14	3333-3340
hotspot 15	3403
hotspot 16	3425-3429
hotspot 17	3446-3797
hotspot 18	3921

Appendix S5: Phylogenetic relationships in *Peperomia* subgenus *Tildenia* obtained from Bayesian inference of the *trnK-psbA* gene region. The topology shown here is a 50% majority rule consensus tree constructed from 417006 Bayesian trees. Posterior probabilities are given above branches. The relationships of species and accessions are grouped in the respective clades I-VII (black star = *P. cuchumatanica*, black triangle = *P. chutanka*; for details see main text).
Clades are discussed in detail in the main text.




outgroup
clade I
clade II
clade III
clade IV
clade V
clade VI
clade VII
P. ocrosensis Pe355
P. querocochana Pe372
P. querocochana Pe566
P. pugnicaudex Pe412
P. radiatinervosa Pe61
P. radiatinervosa Pe81
P. radiatinervosa Pe62
P. radiatinervosa Pe215
P. radiatinervosa Pe209
P. radiatinervosa Pe217
P. radiatinervosa Pe211
P. radiatinervosa Pe121
P. cyclaminoides Pe218
P. cyclaminoides Pe219
P. cyclaminoides Pe114
P. cyclaminoides Pe110
P. cyclaminoides Pe118
P. cyclaminoides Pe117
P. cyclaminoides Pe115
P. cyclaminoides Pe220
P. verruculosa Pe569
P. verruculosa Pe435
P. verruculosa Pe573
P. verruculosa Pe478
P. verruculosa Pe572
P. verruculosa Pe105
P. verruculosa Pe410
P. verruculosa Pe453
P. verruculosa Pe351
P. verruculosa Pe414
P. verruculosa Pe415
P. verruculosa Pe418
P. verruculosa Pe424
P. verruculosa Pe425
P. verruculosa Pe442
P. verruculosa Pe454
P. verruculosa Pe416
P. verruculosa Pe419
P. chutanka Pe436
P. moralesii Pe347
P. gracillima aff. Pe16
P. questionis Pe588
P. occulta Pe49
P. occulta Pe582
P. occulta Pe583
P. mexicana Pe200
P. mexicana Pe45
P. mexicana Pe252
P. bracteata Pe46
P. bracteata aff. Pe255
P. schizandra aff. Pe250
P. macrandra Pe260
P. macrandra Pe201
P. macrandra Pe50
P. schizandra Pe265
P. schizandra Pe21
P. schizandra Pe24
P. schizandra Pe27
P. bracteata Pe528
P. albonervosa Pe22
P. albonervosa Pe26
P. tepoztecoana Pe10
P. monticola Pe257
P. basiradicans Pe11
P. basiradicans Pe4
P. pedicellata Pe37
P. macrandra Pe205
P. macrandra Pe203
P. putlaensis Pe48
P. pedicellata Pe36
P. claytonioides Pe202
P. cavispicata Pe14
P. ampla Pe206
P. ovatopeltata Pe30
P. macrandra Pe13
P. ovatopeltata aff. Pe43
P. ovatopeltata aff. Pe44
P. ovatopeltata aff. Pe41
P. ovatopeltata aff. Pe42
P. bracteata Pe17
P. bracteata Pe18
P. bracteata Pe19
P. elatior Pe12
P. bracteata Pe346
P. bracteata Pe523
P. bracteata Pe529
P. bracteata Pe249
P. bracteata Pe530
P. questionis Pe15
P. ovatopeltata Pe20
P. putlaensis Pe9
P. gracillima aff. Pe23
P. sp. Pe2
P. cavispicata Pe3
P. exclamationis Pe25
P. bracteata Pe33
P. bracteata Pe32
P. bracteata Pe247
P. bracteata Pe28
P. bracteata Pe31
P. bracteata Pe533
P. bracteata Pe534
P. bracteata Pe532
P. bracteata Pe531
P. bracteata Pe527
P. bracteata Pe525
P. bracteata Pe29
P. bracteata Pe256

Appendix S6 **Compilation of the dating and ancestral area reconstruction results. The dating results are given in million years ago (MYA). For BEAST age estimations they are subdivided in results of the full and reduced Tildenia dataset. In the full sampling analysis BEAST estimated two ages for clade V due to reconstructed polyphyly. Relative probabilities of the most probable ancestral areas obtained from LAGRANGE are given in brackets.**

Ages in MYA				Most probable ancestral area		
	BEAST dataset		**r8s**	**DIVA**	**LAGRANGE**	
stem age	full	reduced				
Tildenia	38.69	40.02	40.56	north/central Peru	north/central Peru	(0.521)
clade I	28.05	28.47	24.99	north/central Peru	north/central Peru	(0.7103)
clade II	23.22	22.97	18.92	north/central Peru ,south Peru	south Peru	(0.3058)
clade III	16.02	14.45	12.99	north/central Peru ,south Peru	north/central Peru ,south Peru	(0.8712)
clade IV	13.28	14.09	12.95	north/central Peru	north/central Peru	(0.9999)
clade V	13.28 (16.89)	12.63	11.03	north/central Peru	north/central Peru	(1.0)
clade VI	13.74	13.32	11.44	south Bolivia /north Argentina	north/central Peru, south Bolivia /north Argentina	(0.3564)
clade VII	14.00	12.47	11.15	Mexico	Mexico	(0.78579)
crown age						
Tildenia	28.05	28.47	24.99			
clade I	13.41	12.49	13.94			
clade II	12.70	12.86	10.39			
clade III	13.07	11.68	10.63			
clade IV	12.15	11.90	10.99			
clade V	2.40 (8.77)	1.89	2.32			
clade VI	6.28	5.53	6.19			
clade VII	11.27	10.38	9.33			

CHAPTER 4

Chasing the hare – Evaluating the phylogenetic utility of a nuclear single copy gene region at and below species level

Summary

Background

The increasing number of available genomes does open up almost unlimited prospects for biology in general and molecular phylogenetics in particular. A recent approach took advantage of this data and extracted a set of nuclear genes that occur in one single copy among angiosperms. The utility of one of these genes (*agt1*) as a phylogenetic marker on species level is tested here compared to the performance of several coding and non-coding chloroplast loci that are supposed to be the most applicable for this taxonomic level. As a model group, *Peperomia* subgenus *Tildenia* was chosen since obtaining phylogenetic resolution in a species rich genus, is very challenging, in both plants and animals.

Results

It could be shown that nuclear markers and in particular introns of these genes provide a significantly increased phylogenetic content. One fourth of the *agt1* gene is phylogenetically informative, which is three times higher compared to the potential phylogenetic informative intron and spacer regions of the *trnK-matK-psbA* region. Consequently the *agt1* dataset yields a deeper resolution compared to the applied chloroplast markers. Furthermore, a phylogenetic conflict between the chloroplast and nuclear datasets was detected.

Conclusions

The *agt1* gene is supposed to occur independently from a gene family among angiosperms. This is a great benefit for molecular phylogenetics, since nuclear single copy genes are as far as possible not subject to paralogy. Compared to other nuclear genes that are members of gene families of all sizes, lab effort, such as cloning, can be kept to a minimum. Nuclear single copy genes also provide regions with different phylogenetic content deriving from coding and non-coding parts of different length. Thus, they can be applied on a wide range of taxonomic levels from family down to population level. All of these facts make a very promising tool for molecular phylogenetics.

Introduction

Molecular phylogenetics contributed significantly to reconstruction of a largely resolved 'tree of life' in recent decades (Maddison *et al.* 2007). In flowering plants, the relationships are, with few exceptions, clear from the deepest branches down to the family level (Stevens 2001 onwards, APG III 2009). The most common marker regions used in plant phylogenetics at this time are coding and non-coding sequences from the chloroplast genome and ribosomal gene regions located in the nucleus. Mitochondrial genes are not as widely utilized in plant phylogenetics, but they do play a substantial role for phylogenies of for instance parasitic plants (e.g. Nickrent 2002, Barkman *et al.* 2007) where plastid genomes can be highly modified (dePamphilis and Palmer 1989, Wicke *et al.*, in press.).

The major goal in systematics is to resolve phylogenetic relationships across any level. However, this has been and still is challenging, in particular on genus level and below, since this requires highly variable molecular markers that provide the designated resolution. "The Tortoise and the Hare" series (Small *et al.* 1998, Shaw et al 2005 and 2007) addressed the utility of nuclear and chloroplast loci for low level phylogenetics. The authors noticed an increased resolution with the nuclear gene *Adh*, but the chloroplast markers required much less laboratory effort. Thus, there is a number of promising chloroplast regions identified for low-level applications (Shaw *et al.* 2007). Recently, whole plastid genomes have been used to address evolutionary biological questions (e.g. Jansen *et al.* 2007, Moore *et al.* 2007, Wang *et al.* 2009). Plastid genomes provide multiple genes, and the amplification and direct sequencing of PCR product is easy as a single cell harbors 1000 or more plastids. Chloroplast genomes provide an almost unmatched source of orthologous sequence that is not complicated by gene family duplication and multiple genes can be concatenated to provide a very long sample of orthologous sequence. However, chloroplast markers are tightly linked on a single (typically) non-recombining molecule that usually reflects the maternal lineage only, limiting the generality of chloroplast DNA as the sole source of evolutionary markers at and below species level. In addition, plastid genomes are limited in their variability (e.g. substitution rates/parsimony informative sites) and therefore their utility for molecular low taxonomic level studies may be restricted (Small *et al.* 1998, Duarte *et al.* 2010).

The spacer regions of nrDNA often provide higher variability, but their orthology is merely assumed. Hundreds to thousands copies of those gene regions located in the nucleus make those markers easy to amplify, but their evolution by tandem duplication events results in a gene array more accurately described as a gene family or as paralogs (Alvarez & Wendel 2003, Bailey *et al.* 2003). The paralogous nature of nrDNA can complicate the reconstruction of phylogenetic relationships as it is impossible to determine

orthologous copies from such a large copy number. As a consequence of picking paralogs for phylogenetic reconstruction, artifacts may appear (e.g. Koonin *et al.* 2005).

First attempts to establish nuclear markers, other than ribosomal genes, yielded a substantial number of low copy nuclear genes (LCNG) (Strand *et al.* 1997). LCNG, such as the *ADH*-genes (Sang & Zhang 1999), *pistillata* (Bailey & Doyle 1999), *GPAT* (Tank & Sang 2001), *PRK* (Thomas *et al.* 2006) or *LEAFY* (Kim *et al.* 2008) were applied in numerous studies. They are known to occur either in a single copy or as members of small gene families (Sang 2002) and may not occur in any specific plant lineage. Even though gene families can theoretically be reconciled to reveal the species tree, single loci are mostly preferred for practical reasons as capturing an entire set of genes, including cloning experiments and increased sequencing costs, requires an immense lab effort.

Hughes *et al.* (2006) propose the exploration of nuclear loci other than nrDNA for phylogenetic reconstruction which would require the abandonment of 'universal thinking'. The accelerated variability of nuclear loci compared to other markers provides great potential, but limits the likelihood of identifying universal amplification primers that will function across a wide taxonomic range.

Many different approaches have been used to identify useful nuclear loci in plants, with results varying widely based on computational effort as well as on the quality and amount of preliminary sequence data available for the effort. In recent years, the advent of next generation sequencing technologies (NGS), bioinformatic progress, and publicly available sequence data have facilitated greatly the identification of such loci (Wall *et al.* 2008). These rich sources of information have been utilized by many research groups who identified new nuclear markers for both plant and animal phylogenetics to reveal relationships that could not be resolved by organelle or nuclear ribosomal DNA markers (Fulton *et al.*2002, Wu *et al.* 2006, Whittall *et al.* 2006, Chapman *et al.* 2006, Alvarez *et al.* 2008, Wahlberg & Wheat 2008, Wiegmann *et al.* 2009, Duarte *et al.* 2010). All of them focus in particular on genes that occur in the nucleus in single copy, greatly reducing the problems of paralogy. The present study is based on the approach published in Duarte *et al.* (2010), where a global classification of plant protein coding sequences (Tribes) was used to identify a collection of 959 genes that are represented by exactly one copy in each of four sequenced angiosperm genomes (*Arabidopsis*, *Populus*, *Vitis*, and *Oryza*). This set of genes was called APVO SSCG (shared single copy genes). The *agt1* gene, applied in the present study, is part of that set and the abbreviation nSCG that is used here refers to this approach. Occurring independently all over the nuclear genome in a virtually inexhaustible repertoire in terms of both number and variability, biparentally-inherited single copy loci are promising on different levels, especially when compared to organellar markers. Those benefits make nuclear single copy genes (nSCG) applicable for a broad

range of evolutionary investigation, including systematics and character evolution (e.g. Schultheis & Baldwin 1999), hybridization (e.g. Sang *et al.* 1997), polyploidization (e.g. Doyle *et al.* 2004), biogeography (Lavin *et al.* 2004), origins of domestication (e.g. Nesbitt & Tanksley 2002), and speciation (e.g. Barraclough & Nee 2001). In addition, nSCG are an invaluable tool in developmental biology and comparative genomics as well.

Peperomia (Piperaceae) is one of the ten largest angiosperm genera, with approximately 1,650 species (chapter 3). The phylogenetics and classification of such species-rich clades has long been very challenging. In addition, morphological characters have been shown to be subject to parallel evolution and extreme reduction, resulting in a paucity of synapomorphies (Wanke *et al.* 2006, Samain *et al.* 2009). Moreover, speciation within *Peperomia* has likely happened comparatively recently (chapter 3); because of this, reconstructed phylogenies often lack resolution at the species and population level (Bradley 2002). Recent backbone phylogenies had to accumulate a significant amount of molecular characters to overcome the lack of variability (Wanke *et al.* 2006, Samain *et al.* 2009). Therefore, a species-level study in *Peperomia* subgenus *Tildenia* makes an ideal candidate to test the performance of a nSCG region including its introns to variable chloroplast markers suggested by Shaw *et al.* (2005, 2007) in a comparative approach.

Methods

Sampling strategy

Based on chapter 1 and 2 a phylogenetically representative subset (29 out of 59 species) of *Peperomia* subgenus *Tildenia* was sampled (Appendix 1). As outgroup taxa, *Peperomia pinoi* aff. Pe474, closely related to this subgenus, was chosen (Wanke *et al.* 2006, Samain *et al.* 2009). For a comprehensive approach to compare the *agt1* gene with the *trnK* intron, *matK* gene and the *trnK-psbA* spacer region, 63 accessions including the outgroup species were sampled. In addition, the *agt1* gene was compared to two additional chloroplast gene clusters containing both introns and spacers (*ndhF-rpl32-trnL* and *psbA-trnH*). A restricted set of 26 accessions including outgroup was utilized for this.

Marker selection

The starting point for the present study were 959 genes identified by Duarte *et al.* (2010), that are shared in single copy in the annotated genomes of *Arabidopsis thaliana*, *Populus trichocarpa*, *Vitis vinifera* and *Oryza sativa* (APVO). The authors used a high throughput comparative proteomic approach to identify genes that occur in all of the four genomes in only one single copy. This comprehensive approach is assumed to be the most valuable to obtain candidate markers for any angiosperm of interest, because the genes captured this way are putatively present in most (or all) angiosperms and thus potentially suitable useful as phylogenetic markers.

Starting from potential nSCGs compared by Duarte *et al.* (2010), ESTs (Expressed Sequence Tags) obtained from the MAGIC database from the Ancestral Angiosperm Genome Project (http://ancangio.uga.edu/) were screened for homologues of other representatives of basal angiosperm lineages. For those representatives, the number and appropriate overall length for amplification and sequencing were checked in fully sequenced genomes. Comparison of EST data and further characterization was done using PlantTribes (http://fgp.bio.psu.edu/tribedb/index.pl, Wall *et al.* 2008) and TIGR Plant Transcript Assemblies (http://plantta.jcvi.org, Childs *et al.* 2007). Initial primer sets were designed for this particular region using EST data from other basal angiosperm lineages to subsequently amplify and sequence the gene region in a RT-PCR approach for *Piper nigrum* and *Peperomia prostrata* (4474-390F: ACCAGGGAGGAACCATCTCTTTG; 4474-1530R: TTYTTCARMCCCCATGCTTC). These sequences were used to build primers that are more specific for Piperaceae to amplify and sequence different *Peperomia* accessions.

A single primer was designed in a highly conserved 5' region of the gene (Pe-4474-1800F: TTCTTTGAYTGGAATGACTACTTGA) which was applied in a 3'-RACE (3´ RACE System for Rapid Amplification of cDNA Ends, Invitrogen Corporation) for *Peperomia cyclaminoides* Pe114. Based on the resulting sequence, primers at the outermost end of the gene were generated and have been applied to a broad set of *Peperomia* accessions (Appendix 2).

DNA isolation, RNA isolation, cloning, amplification, RT-PCR, sequencing and alignment

DNA was isolated from silica dried material using the CTAB method described in chapter 1. Most of the sequences of the *trnK-matK-psbA* gene region are obtained from chapter 3. Amplification of the remaining chloroplast sequences follows the procedure presented in chapter 1. Primers used for amplification and sequencing of the chloroplast markers are listed in Appendix 2. For the nuclear marker, both 25 µl and 50 µl reactions were run containing between 0.5 and 4 µl DNA template (100 to 200 ng) for a 50 µl reaction. A 'master mix' for a 50 µl reaction contained 8 µl dNTP (Roth, 1.25 mM each), 5 µl red Taq-buffer for high yields (PeqLab), 1.5 µl $MgCl_2$ (25 mM), 1 µl of each primer (50 pmol/µl) and 0.5 µl of Taq DNA polymerase (PeqLab). Water was added to obtain a total reaction volume of 50 µl. For amplification of the *agt1* a standard PCR-program for low concentration of target DNA was modified. The program starts with an initial denaturation step at 94 °C for 2 min, followed by 45 cycles of denaturation at 96 °C for 45 sec, annealing at 51 °C for 30 sec and elongation at 72 °C for 90 sec, and a final elongation 72 °C for 7 min. Annealing temperature was adjusted to the melting point of the primers. PCR was run on a T3 Thermocycler (Biometra). After gel electrophoresis through a 1.2 % agarose gel, the PCR products were purified using a gel extraction kit (Macherey&Nagel). Some of the PCR products required an additional cloning step. In this case a T/A Cloning Kit (Genaxxon BioScience) was applied. A ligation reaction was set up with 2-4 µl PCR-product, 1µl of each ligase buffer, ligase and vector and filled up with water to a total reaction volume of 10 µl. Three to nine clones were arbitrarily chosen and directly amplified via colony PCR using M13 primers under the following conditions: initial denaturation at 95°C for 2 min, denaturation at 95°C for 1 min, annealing at 55°C for 1 min, elongation at 72°C for 1.5 min and a final elongation at 72°C for 10 min. This PCR was run with 36 cycles and the products were directly sequenced after purification.
RNA was isolated using peqGold Plant RNA Kit (PeqLab) following the manufacturer's instructions. A 3' RACE kit (Invitrogen Life Science) was used following the manufacturer's protocol. For RT-PCR the Access RT-PCR System (Promega) was used following the manufacturer's instructions.

Direct sequencing was conducted either using a Beckman Coulter CEQ DTCS Quick Start Kit (Beckman Coulter) with the CEQ 8000 sequencer or using Macrogen's sequencing service (Macrogen Inc., Korea). Sequences were edited and aligned manually using PhyDE (Müller *et al.* 2005). Regions of uncertain homology (e.g. long monobase repeats) were excluded from all subsequent analyses.

Due to the length of the *atg1* gene, DNA was not amplified in one single reaction but in three parts (sections) with substantial overlap (50 to 150 bp) to ensure efficient amplification. The ESTs and cDNA sequences of basal angiosperms mentioned above, which were available to generate initial amplification primers, did not cover the entire region. The 3'-ends of the ESTs usually reached a sequence section equivalent to exon IV in *Arabidopsis thaliana* (Figure 1) making a RACE approach necessary to include full length gene information (intron IV and exon V).

Very few PCR products (approximately 10 %) revealed differences in length. If there were multiple bands visible on the gel, they were cut out individually. The middle section yielded sequences without any length mutations, whereas either the first or the third section or both sections required a cloning step for several accessions. The three sections of those accessions had to be assembled manually to gain a full sequence. In the worst case, both, the first and the third part of an accession yielded up to three different 'types' of copies. If this occurred, it was determined phylogenetically which fragments originate from the same copy. This method allowes to avoid artifacts due to incorrect sequence combinations.

However, most of the detected multicopy accessions were monophyletic in the phylogenetic tree and thus, a copy could randomly be chosen for the final calculation. For the few accessions that turned out polyphyletic, a copy of each 'type' was left in the dataset and was distinguished by 'type I' and 'type II'. All major clades of subgenus *Tildenia* contained at least one accession that required cloning steps; thus multiple copies are not unique to a particular lineage

Phylogenetic analyses

Several mostly small regions of uncertain sequence homology (hotspots) had to be excluded from the different data matrices (Table 1).

Indel matrices were calculated using a “simple indel coding” approach (SIC, Simmons and Ochoterena, 2000). This indel matrix was generated automatically by SeqState (Müller 2005), which provides an IndelCoding tool. Substitution models for Bayesian inference were determined using jModelTest (Posada 2008). For the *agt*1 dataset the general time reversible model of nucleotide substitution and site-specific rate categories following a gamma distribution (GTR+I+Γ) was assigned as the best matching model considering the Akaike information criterion (AIC). For the three chloroplast datasets, the GTR+Γ model was determined to match best. Bayesian MCMC inferences were performed employing MrBayes v3.1 (Ronquist and Huelsenbeck 2003) assuming the respective substitution model mentioned above.

The Bayesian inference was applied with four Markov chains running simultaneously for 4 million generations, saving trees each 100 generations. The burn-in was individually set for each analysis between 5 and 20 % after determining stationarity of each run with Tracer v1.5 (Rambaut and Drummond 2005). Runs of very poor quality have been removed from the calculation of the consensus trees and posterior probabilities, but for each calculation, at least ten runs were assembled to generate the consensus tree.

Furthermore, the degree of homoplasy of each dataset both with and without indels was assessed using a parsimony ratchet approach. Command files for Maximum Parsimony (MP) analyses were created using PRAP (Müller 2004) and executed in PAUP*4b10 (Swofford 2002). Topologies were gained using heuristic search strategy and 10 random addition cycles of 200 iterations each with a 25 % upweighting of the characters in the iterations. For compiling and drawing all trees TreeGraph 2 (Stöver and Müller 2010) was employed.

Sequence statistics for the gene sections of the different marker were obtained utilizing SeqState (Müller 2005). The nonparametric Shimodaira–Hasegawa (SH) test (1999) was performed in PAUP*4b10 (Swofford 2002) with full optimization to evaluate the topologies obtained by the different genetic markers against each other. The SH test simultaneously compensates for posteriori hypotheses of multiple alternative topologies by adjusting the expected difference in log-likelihood values. To comply with the congruence of sampling in the reduced datasets the multiple copies were restricted to a single accession in the *agt1* datasets. In case of conflict between two markers ($p \leq 0.05$) the test was repeated with a manually changed topology to determine the source of conflict. In the process, one hypothesis (topology) was stepwise adjusted to phylogenetic results of the conflicting marker to evaluate the effects of single clade position changes.

Table 1. Regions of uncertain sequence homology (hotspots) excluded from the different datasets.

	agt1	*trnK-matK-psbA*	ndhF-rpl32-trnL	*psbA-trnH*
alignment position	1504-1508 1600-1612 1948-1952 2088-	401-406 429-439 487-493 2397-2420 2560-2570 2739-2746 2822-2828 3167-	315-326 353-360 419-440 453-457 676-690 873-878 1027-1035 1433-1445 1710-	58-78 84-89 186-204 400-

Results

The *atg1* gene studied here is an ortholog of At2G13360 in *Arabidopsis thaliana*, where it is known to catalyze the Alanine:Glyoxylate Aminotransferase reaction located in peroxisomes (Liepman *et al.* 2003). It was identified to be shared between four angiosperm genomes (*Arabidopsis*, *Populus*, *Vitis*, and *Oryza*) as a nSCG (Duarte *et al.* 2010). This region was chosen as an example to test the utility of nSCG for low level phylogenies in comparison to eight widely studied chloroplast markers (Shaw *et al.* 2005 and 2007). The *agt1* region was amplified, sequenced, and aligned for 62 accessions covering 33 out of the 59 species of *Peperomia* subgenus *Tildenia* resulting in a 2088 bp dataset without hotspots (Table 2). An identical dataset was generated for the *trnK* intron, the *matK* gene and the *trnK-psbA* spacer resulting in a 3049 bp aligned dataset (without hotspots). To allow further exploration of the utility of the *agt1*, additional chloroplast markers for a sub-sample of 26 representative accessions were added. These markers comprised the *psbA-trnH* spacer plus short parts of flanking genes (315 bp, without hotspots), as well as the the *ndhF-rpl32-trnL* gene region, consisting of a partial sequence of the *ndhF* gene (204 bp), the *ndhF-rpl32* spacer (620 bp), the *rpl32* gene (158 bp) and partial sequence of the *rpl32-trnL* spacer (848 bp). The *ndhF-rpl32* spacer, *rpl32* gene and *rpl32-trnL* spacer were co-amplified and yielded an aligned dataset of 1830 bp. All markers are among the most variable loci for phylogenetic studies according to Shaw *et al.* (2005, 2007).

An important feature of *atg1* in *Peperomia* is that PCR amplicons typically were resolved as a single sharp target sized band on agarose gels and, for most samples, could be sequenced directly without cloning. For polymorphic bases the IUPAC Ambiguity Codes were used. Very few accessions showed a single band in the agarose gel ,but sequencing revealed differences in length comprising 1 to 10 basepair. The majority of detected differences among clones were restricted to one single intron.

Table 2. **Sequence statistics of the applied gene regions after exclusion of regions of uncertain sequence homology (hotspots). The values has been calculated with SeqState (Müller, 2004).**

	agt1 full			agt1 reduced		
	coding	non-coding	total	coding	non-coding	total
total characters	1088	1000	2088	1088	847	1935
indels	0	165	165	0	96	96
length range	906-1088	552-739	1542-1827	906-1088	552-729	1548-1817
mean length	1,039.629	684.613	1,724.242	1,038.040	680.600	1,718.640
ti/tv ratio	1.641	1.081	1.193	1.497	1.057	1.152
divercence %	2.288	8.087	4.525	2.215	7.498	4.253
GC %	45.284	29.965	39.201	45.322	29.997	39.253
variable % (total)	19.945 (217)	46.100 (462)	32.471 (679)	13.971 (152)	39.669 (337)	25.220 (489)
constant % (total)	80.055 (871)	53.900 (538)	67.529 (1409)	86.029 (936)	60.331 (510)	74.780 (1446)
uninformative % (total)	9.191 (100)	19.500 (196)	14.128 (296)	8.548 (93)	24.793 (211)	15.659(304)
informative % (total)	10.754 (117)	26.600 (266)	18.343 (383)	5.423 (59)	14.876 (126)	9.561 (185)
PIC/aligned characters	0.108	0.266	0.183	0.054	0.149	0.096

	matK-trnK-psbA full			matK-trnK-psbA reduced		
	coding	non-coding	total	coding	non-coding	total
total characters	1609	1440	3049	1606	1267	2873
indels	11	62	73	7	38	45
length range	1544-1589	1140-1346	2691-2893	1544-1589	1151-1231	2698-2789
mean length	1,552.339	1,203.129	2,755.468	1,553.680	1,204.360	2,758.040
ti/tv ratio	1.169	1.151	1.124	1.180	1.212	1.187
divercence %	1.944	2.444	2.159	1.905	2.431	2.103
GC %	32.811	33.753	33.222	32.824	33.591	33.159
variable % (total)	15.848 (255)	16.111 (232)	15.972 (487)	11.644 (187)	13.970 (177)	12.496 (359)
constant % (total)	84.152 (1354)	83.889 (1208)	84.028 (2562)	88.356 (1419)	86.030 (1090)	87.504 (2514)
uninformative % (total)	7.520 (121)	7.292 (105)	7.412 (226)	6.040 (97)	7.024 (89)	6.509 (187)
informative % (total)	8.328 (134)	8.819 (127)	8.560 (261)	5.604 (90)	6.946 (88)	5.987 (172)
PIC/aligned characters	0.083	0.088	0.086	0.056	0.069	0.060

	ndhF-rpl32-trnL reduced			psbA-trnH reduced		
	coding	non-coding	total	coding	non-coding	total
total characters	362	1258	1620	29	286	315
indels	5	93	98	0	38	38
length range	287-361	828-990	1171-1334	29-29	126-187	141-216
mean length	342.080	917.880	1,259.960	29.000	161.875	189.667
ti/tv ratio	1.342	1.285	1.298	0.048	1.057	1.063
divercence %	2.004	2.844	2.611	0.872	2.542	2.273
GC %	31.952	22.718	25.225	27.586	31.931	35.863
variable % (total)	12.155 (44)	12.719 (160)	12.593 (204)	6.897 (2)	11.189 (32)	10.794 (34)
constant % (total)	87.845 (318)	87.281 (1098)	87.407 (1416)	93.103 (27)	88.811 (254)	89.206 (281)
uninformative % (total)	5.801 (21)	6.996 (88)	6.729 (109)	3.449 (1)	6.993 (20)	6.667 (21)
informative % (total)	6.354 (23)	5.723 (72)	5.864 (95)	3.448 (1)	4.196 (12)	4.127 (13)
PIC/aligned characters	0.064	0.057	0.059	0.034	0.042	0.037

Structure and characterization of *agt1*

For designing initial primers for this region, Sanger and 454 (Roche) ESTs that derived from several basal angiosperms (*Amborella*, *Nuphar advena*, *Liriodendron tulipifera*, *Persea americana*) were used (Appendix 2). These yielded sequences for *Piper nigrum* and *Peperomia prostrata*, which were used to build primers that are specific for Piperaceae and were used for further amplifications from *Peperomia* species. For a more thorough characterization of this gene in this genus, *Peperomia cyclaminoides* was used to perform a 3'-RACE and to determine intron-exon boundaries.

In *Peperomia*, the *agt1* gene comprises five coding regions (exons) separated by four intronic sections with a total length of 1542 to 1827 bp. In comparison to *agt1* in *Arabidopsis thaliana*, the *Peperomia* gene contains an additional intron, which is located in the second exon and has a length of 211-390 bp (Figure 1). This intron is also present in the genomic sequences of *Populus trichocarpa, Medicago truncatula, Solanum lycopersicon, Oryza sativa, Carica papaya*, as well as in all *Peperomia* species generated in the present study. Hence, it can be inferred that it was lost during the diversification of Brassicales. This additional non-coding section that splits exon II into two parts was named intron X, and the two resulting exons that derive from exon II were named exon IIa and exon IIb (Figure 1). In *Peperomia*, the *agt1* exons are highly conserved in length and lack any indels. In contrast, intron X was the most variable in length among the respective four introns, showing a length range of 552-739 bp.

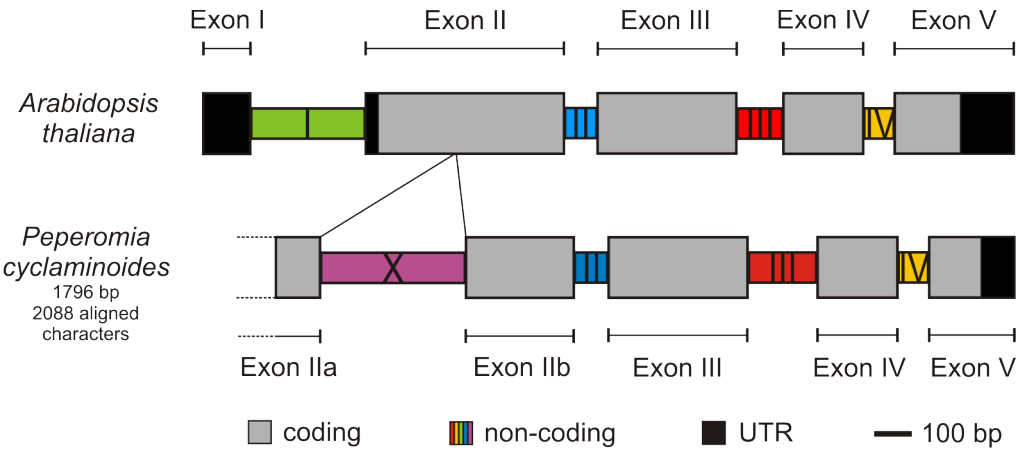


Figure 1. **The Alanine: Glyoxylate-Aminotransferase (*agt1)* gene model in *Arabidopsis thaliana* and in *Peperomia cyclaminoides***

Variability of *agt1* versus the chloroplast regions

To characterize the markers applied in this study in more detail, several variability parameters have been captured for both coding and non-coding regions and for full as well as reduced sampling (Table 2, Figure 2). Regarding the *agt1* full sampling dataset, introns and exons are roughly equally represented (introns 1000 bp, exons 1088 bp, see Table1). Coding parts possess 20 % variable characters (VC) and 11 % parsimony informative characters (PIC), whereas non-coding parts contain 46 % VC and 27 % PIC. This means the content of PIC varies dramatically among different sections of the gene and in non-coding parts it is twice as high as in coding parts. In total, one fifth of the characters in the *agt1* gene are parsimony informative.

The *trnK-matK-psbA* region does not vary significantly among coding and non-coding regions (Table 1). Compared to the *agt1* with the identical sampling this region shows less than half of the ratio of VC and PIC . Thus, the *agt1* is much more effective in yielding PIC per sequenced bp than the widely applied *trnK-matK-psbA* region. Furthermore, the nuclear gene segments provide different amounts of variability, which make them useful for many taxonomic levels.

The reduced sampling of all utilized markers yields a similar pattern (Table 1). In total, the percentages of both variable sites (25 %) and PIC (15 %) of *agt1* are much higher than those of the chloroplast markers. However, it has to be noted that the VC percentage of the reduced sampling is 7 % lower than that of the full sampling and that there are only half of the PIC in the reduced dataset. In contrast, the *trnK-matK-psbA* does not possess large differences between reduced and full sampling datasets. The decrease of VC in the reduced sampling suggests that the potential of *agt1* is not exhausted and deeper sampling would likely increase available variability and thus phylogenetic resolution.

The variabilities of the different gene regions are graphically displayed in Figure 2. It is remarkable that the chloroplast markers are highly similar in their portion of variability per total sequenced base pairs. Thus, the number of PIC is directly proportional to the number of sequenced bp and thus on this phylogenetic level marker selection does not seem to be crucial in terms of phylogenetic content. Furthermore, non-coding regions of all chloroplast markers are just as informative as non-coding regions on this phylogenetic level. To look for potential saturation of the markers, the retention index (RI) was calculated with and without indel-coded length mutations. Generally, for the chloroplast markers the RI does not differ between the datasets that include indels and those without indels (Table 3). The RI values of both *trnK-matK-psbA* datasets (full sampling including indels: RI=0.873, reduced sampling including indels: RI=0.828) as well as *ndhF-rps32-rnL* dataset (reduced sampling including indels RI=0.836) indicate low homoplasy.

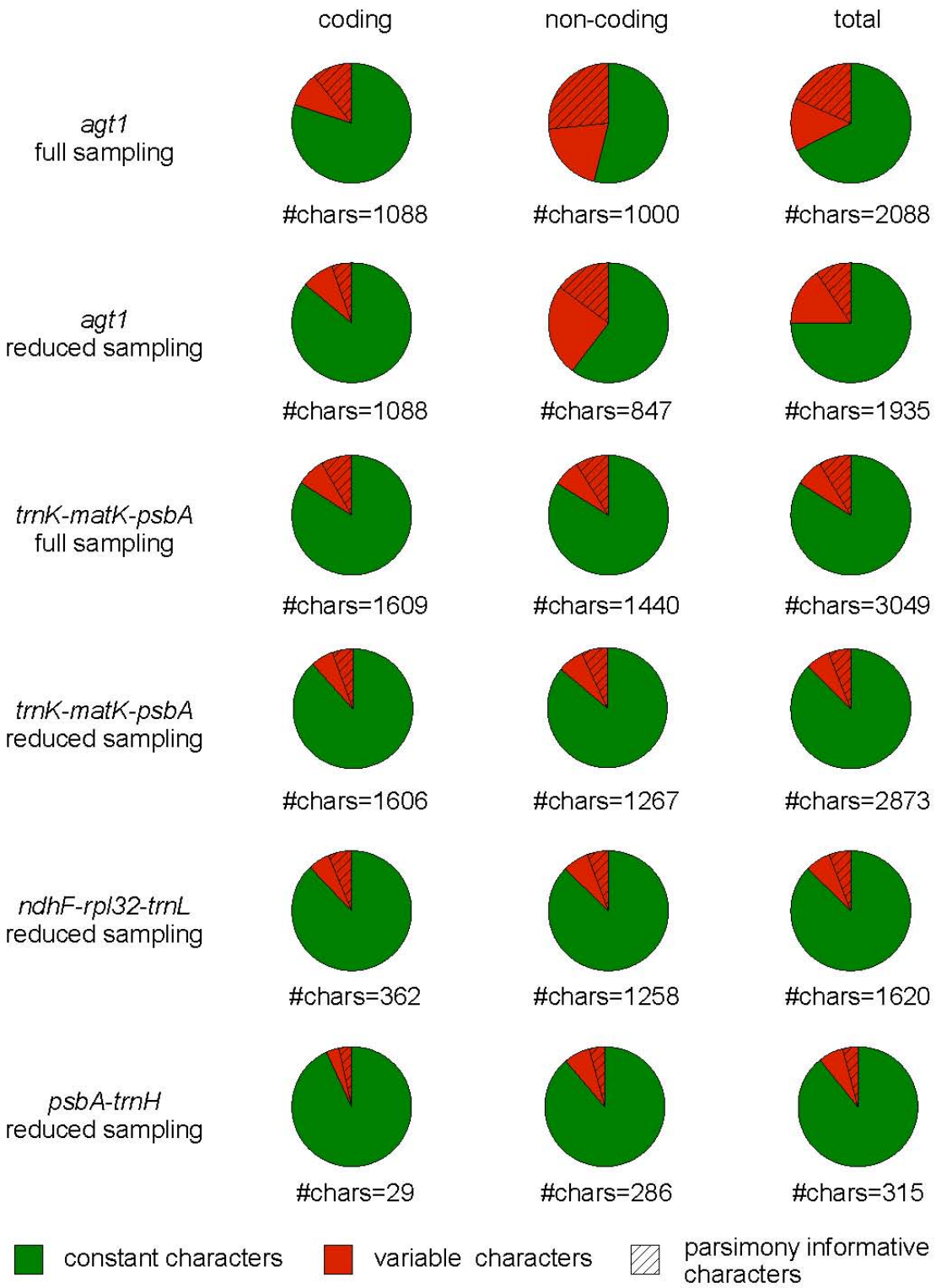


Figure 2. **Characteristics of utilized markers. Portions of constant (green), variable (red) and parsimony informative characters (dashed) for coding and non-coding parts of all applied markers in this study were calculated with SeqState (Müller, 2004) (#chars = number of characters).**

The homoplasy of *agt1* full sampling (including indels, RI=0.802) is little homoplastic as well. Only the RI values of *psbA-trnH* (reduced sampling including indels RI=0.697) and *agt1* reduced sampling datasets (including indels: RI=0.658) datasets are a little lower, which suggests that they are more homoplastic and likely closer to saturation. Considering the RI, both *trn*K-*mat*K-*psb*A and *ndh*F-*rpl*32-*trn*L can be regarded as little homoplastic. The RI of the different *agt1* datasets differs and is lower in the reduced sampling. Consequently, a smaller sampling is more homoplastic than a denser sampling with the same breadth. This fact is congruent with the phylogenies of the *agt1* gene, resulting in less resolution and support in the reduced dataset. This leads to the conclusion that a high variability as detected for the *agt1* gene requires a dense sampling to keep the risk for artifacts low. Summarizing, a quarter of the total *agt1* dataset is parsimony informative, while having a low homoplasy level at the same time. All calculations were based on nucleotide substitutions and without an indel coding, as RIs of all data sets revealed this to be less homoplastic than the data sets containing indels (Table 3).

Table 3 **Statistics based on a Maximum Parsimony Ratchet analysis showing the retention index (RI) as a homoplasy value (n.a.= not available; hotspots excluded).**

		total characters (with indels)	Trees found (with indels)	Steps (with indels)	RI (with indels)
agt1 full	coding	1088 (1088)	1253 (n.a.)	405 (n.a.)	**0.827 (n.a.)**
	non-coding	1000 (1192)	1721 (1539)	978 (1205)	**0.799 (0.794)**
	total	2088 (2280)	232 (362)	1395 (1622)	**0.802 (0.798)**
agt1 reduced	coding	1088 (1088)	206 (n.a.)	260 (n.a.)	**0.742 (n.a.)**
	non-coding	847 (971)	323 (55)	613 (746)	**0.636 (0.628)**
	total	1935 (2059)	50 (64)	876 (1010)	**0.667 (0.658)**
matK-trnK-psbA full	coding	1609 (1621)	158 (172)	448 (461)	**0.882 (0.883)**
	non-coding	1440 (1554)	39 (85)	422 (518)	**0.879 (0.877)**
	total	3049 (3174)	1007 (1356)	881 (990)	**0.874 (0.873)**
matK-trnK-psbA reduced	coding	1606 (1614)	18 (15)	303 (312)	**0.840 (0.383)**
	non-coding	1267 (1361)	9 (28)	287 (343)	**0.837 (0.831)**
	total	2873 (2974)	12 (21)	587 (651)	**0.832 (0.828)**
ndhF-rpl32-trnL reduced	coding	362 (367)	2 (2)	58 (63)	**0.962 (0.964)**
	non-coding	1258 (1583)	10 (8)	246 (382)	**0.822 (0.823)**
	total	1620 (2150)	1 (65)	305 (448)	**0.847 (0.836)**
psbA-trnH reduced	coding	29 (29)	1 (n.a.)	2 (n.a.)	**1.000 (n.a.)**
	non-coding	286 (320)	810 (28)	46 (100)	**0.727 (0.714)**
	total	315 (399)	1044 (38)	49 (103)	**0.696 (0.700)**

Phylogenetic output

To obtain insights into *agt1*'s phylogenetic performance in terms of topology, resolution and support, identical phylogenetic analyses with identical sampling and sub-sampling for all markers were performed (Figure 3). For simplicity, the clades have been named with numbers.

All phylogenetic trees obtained from the different markers possess species from clade I and II as the first diverging branches. In *agt1* trees, four clades out of seven are monophyletic and maximal supported in all phylogenetic trees obtained in this study (clade II, IV, VI and VII) (Figure 3). Clade I is polyphyletic in the *agt1* tree, but monophyletic in any of the chloroplast markers. The latter result is also found when all datasets are combined (7243 aligned characters). A new clade is formed in the *agt1* phylogeny, consisting of *P. cerrateae*, *P.* sp. Pe411, *P. pugnicaudex*, and *P. chutanka*. Clade VI is sister to this clade, which is supported with 0,97 PP by the full sampling and with 0,89 PP by the reduced. Thus, clade III and clade V are not monophyletic in the nuclear-based tree and neither in the tree resulting from *ndhF-rps32-trnL*. In the *trnK-matK-psbA* tree, *P. cerrateae* was part of clade V, *P.* sp. Pe411 and *P. pugnicaudex* formed clade III and the position of *P. chutanka* could not be resolved. In general, major differences between the nuclear and chloroplast markers become obvious on clade level. In summary, *agt1* yields a deeper resolution compared to any individual or combined chloroplast dataset, e.g. by forming a new sister clade to clade VI.

Statistical evaluation of phylogenetic signals between datasets was executed using SH tests (Shimodaira–Hasegawa 1999). These tests reveal a significant conflict between the chloroplast markers and the nuclear *agt1* gene, expressed by the rejection of all respective topology/dataset combinations (Appendix 3). The only exception of this pattern is the combination of the *ndhF-rpl32-trnL* topology and the *agt1* dataset (p=0,070). *The trnH-psbA* spacer was not used for this test due to insufficient phylogenetic resolution. In accordance with the result of Shaw *et al.* (2005), this finding states the inappropriateness of this region for phylogenetic studies on low taxonomic level.

To determine the source of conflict between the nuclear and chloroplast markers, 10 alternative hypotheses were tested by changing the topologies of the reduced *agt1* and *trnK-matK-psbA* datasets. In most cases one single clade was replaced according to its phylogenetic position in the opposed topology. Additionally, clades that appear polyphyletic in one topology were constrained to be monophyletic and placed on different positions. All alternative hypotheses resulted in significant rejection by the compared datasets.

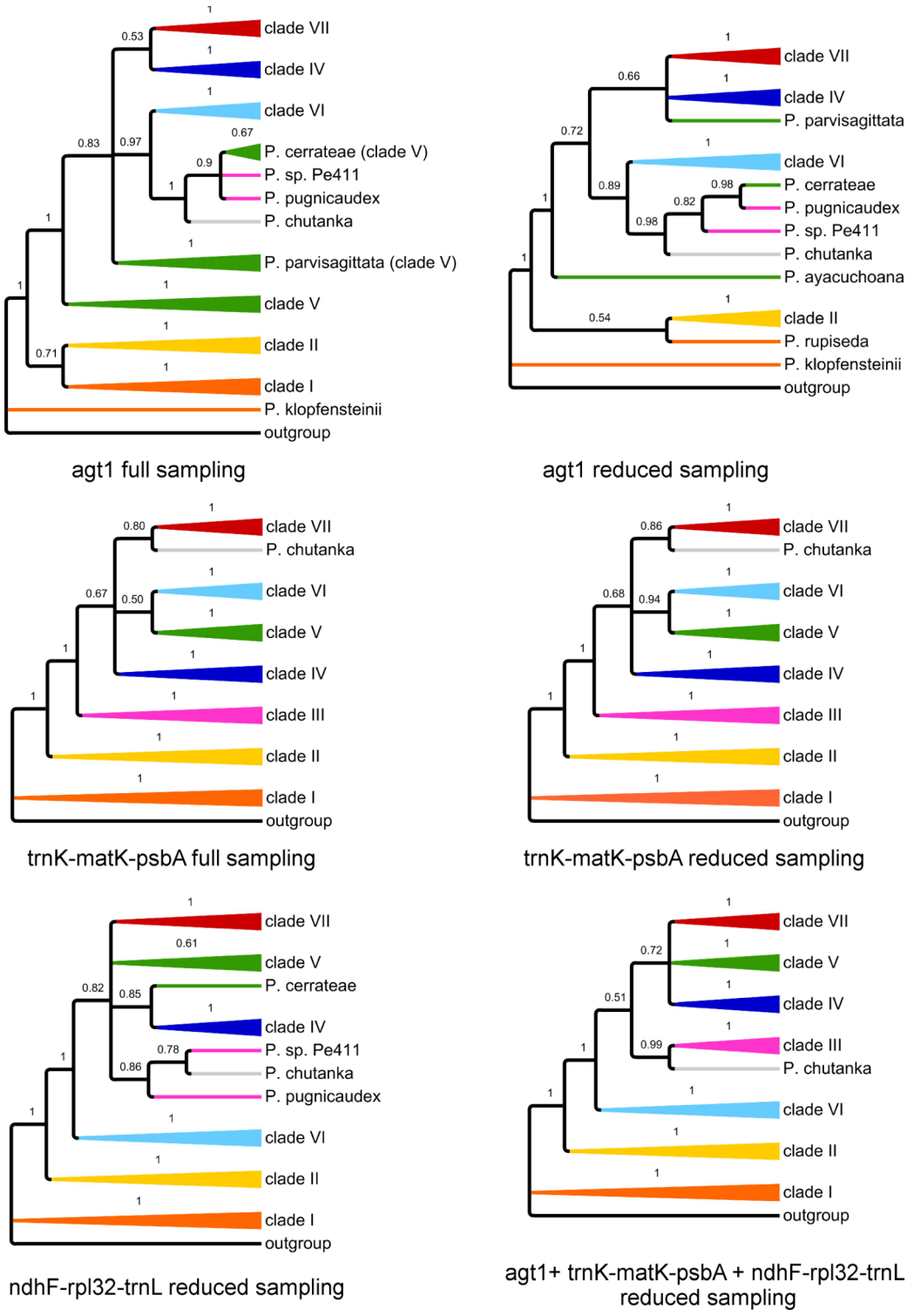


Figure 3. **Schematical summary of topologies based on Bayesian inference: *agt1* and *trnK-matK-psbA* are shown for both full and reduced sampling, and *ndhF-rpl32-trnH* for reduced sampling. Clades were named based on the phylogentic results of chapter 3. Full sampling comprises a 63 accession dataset, reduced sampling comprises 26 accessions.**

The alternative topologies and the SH-values of the accordant combinations are summarized in Appendix 3. The results indicate a general incongruence of genetic signal of the nuclear and chloroplast genome. The rejection of the alternative hypothesis indicate that the conflict is not caused by a single subset of species or clade, but is reflected in multiple differences of genetic signals between the nuclear versus the chloroplast gene regions. Such a pattern might be expected in the case of an early hybridization event in the evolution of the subgenus.

The combination of *agt1*, *trnK-matK-psbA*, *ndhF-rpl32-trnL*, and *trnH-psbA* data set (7243 total characters) yields a topology possessing all of the monophyletic clades identified by *trnK-matK-psbA* with maximal support. In addition to this, the position of *P. chutanka* is determined in this phylogeny as sister to clade III, which is supported with 0.99 PP. A phylogeny of *trnH-psbA* alone did not yield any resolution (results not shown); nevertheless, these data were included into the combined data set to enlarge the amount of sequence data.

The resolution of the two full sampling phylogenies (Figure 4, Figure 5) was measured using the normalized consensus fork index (CFI), which divides the number of nodes found in a strict consensus tree by the number of terminal taxa (Colless, 1980). To increase the informative value of this index with respect to resolution capacity of the respective molecular markers only well-supported nodes (≥ 0,95 PP) were included here for calculation. According to this, the *agt1* yields a considerably higher total resolution (CFI = 0.71) than the *trnK-matK-psbA* cluster (CFI = 0.56). Generally, the *agt1* resolution is much higher within the clades and on population level, which is very obvious for *P. peruviana*, *P. cerrateae*, *and P. radiadinervosa* as well as within clade IV and VI (Figure 4). Furthermore, the *agt1* phylogeny reveals that *P. cyclaminoides* and *P. verruculosa* are not monophyletic and *P. bracteata* is a polyphyletic species. Clade VII shows better resolution of the basal branches with the chloroplast dataset (Figure 5), whereas the nucleus resolves relationships better in derived parts of the tree. The chloroplast suggests *P. gracillima* aff. to be the first branch, followed by *P. schizandra* to be sister to the rest of this clade. The nucleus, however, reveals a well-resolved subclade comprising *P. elatior* at the first branch, followed by *P. ovatopeltata* aff., *P. questionis* and *P. cavispicata*. Furthermore, in the nuclear phylogeny, *P. exclamationis* can be found as sister to *P. schizandra*.

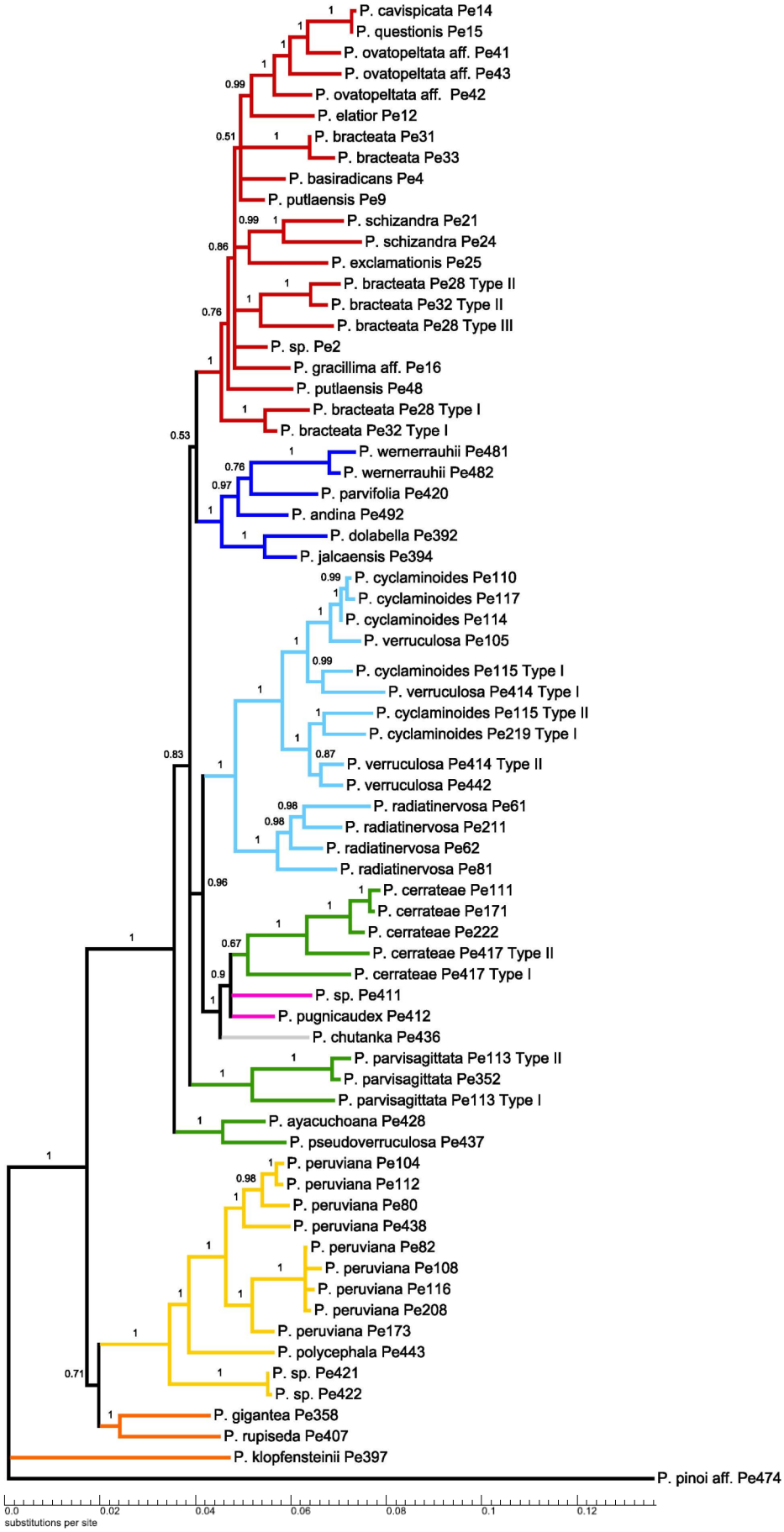

P. cavispicata Pe14
P. questionis Pe15
P. ovatopeltata aff. Pe41
P. ovatopeltata aff. Pe43
P. ovatopeltata aff. Pe42
P. elatior Pe12
P. bracteata Pe31
P. bracteata Pe33
P. basiradicans Pe4
P. putlaensis Pe9
P. schizandra Pe21
P. schizandra Pe24
P. exclamationis Pe25
P. bracteata Pe28 Type II
P. bracteata Pe32 Type II
P. bracteata Pe28 Type III
P. sp. Pe2
P. gracillima aff. Pe16
P. putlaensis Pe48
P. bracteata Pe28 Type I
P. bracteata Pe32 Type I
P. wernerrauhii Pe481
P. wernerrauhii Pe482
P. parvifolia Pe420
P. andina Pe492
P. dolabella Pe392
P. jalcaensis Pe394
P. cyclaminoides Pe110
P. cyclaminoides Pe117
P. cyclaminoides Pe114
P. verruculosa Pe105
P. cyclaminoides Pe115 Type I
P. verruculosa Pe414 Type I
P. cyclaminoides Pe115 Type II
P. cyclaminoides Pe219 Type I
P. verruculosa Pe414 Type II
P. verruculosa Pe442
P. radiatinervosa Pe61
P. radiatinervosa Pe211
P. radiatinervosa Pe62
P. radiatinervosa Pe81
P. cerrateae Pe111
P. cerrateae Pe171
P. cerrateae Pe222
P. cerrateae Pe417 Type II
P. cerrateae Pe417 Type I
P. sp. Pe411
P. pugnicaudex Pe412
P. chutanka Pe436
P. parvisagittata Pe113 Type II
P. parvisagittata Pe352
P. parvisagittata Pe113 Type I
P. ayacuchoana Pe428
P. pseudoverruculosa Pe437
P. peruviana Pe104
P. peruviana Pe112
P. peruviana Pe80
P. peruviana Pe438
P. peruviana Pe82
P. peruviana Pe108
P. peruviana Pe116
P. peruviana Pe208
P. peruviana Pe173
P. polycephala Pe443
P. sp. Pe421
P. sp. Pe422
P. gigantea Pe358
P. rupiseda Pe407
P. klopfensteinii Pe397
P. pinoi aff. Pe474
0.0
0.02
0.04
0.06
0.08
0.1
0.12
substitutions per site

Figure 4 (previous page). **Phylogram of *agt1* full dataset based on a Bayesian inference with relative substitution rates using the GTR+G+I model and posterior probabilities (PP) plotted above the branches. Clades are colored according to Figure 3. In accessions that are represented in the tree by different 'types', multiple copies were detected that are not monophyletic.**


P. bracteata Pe28
P. bracteata Pe31
P. bracteata Pe32
P. bracteata Pe33
P. basiradicans Pe4
P. putlaensis Pe48
P. exclamationis Pe25
P. species Pe2
P. putlaensis Pe9
P. ovatopeltata aff. Pe41
P. cavispicata Pe14
P. questionis Pe15
P. elatior Pe12
P. ovatopeltata aff. Pe42
P. ovatopeltata aff. Pe43
P. schizandra Pe21
P. schizandra Pe24
P. gracillima aff. Pe16
P. chutanka Pe436
P. cyclaminoides Pe110
P. cyclaminoides Pe117
P. cyclaminoides Pe114
P. cyclaminoides Pe115
P. cyclaminoides Pe219
P. verruculosa Pe414
P. verruculosa Pe442
P. verruculosa Pe105
P. radiatinervosa Pe211
P. radiatinervosa Pe62
P. radiatinervosa Pe61
P. radiatinervosa Pe81
P. cerrateae Pe111
P. cerrateae Pe171
P. cerrateae Pe222
P. cerrateae Pe417
P. parvisagittata Pe113
P. parvisagittata Pe352
P. ayacuchoana Pe428
P. pseudoverruculosa Pe437
P. wernerrauhii Pe481
P. wernerrauhii Pe482
P. andina Pe492
P. parvifolia Pe420
P. dolabella Pe392
P. jalcaensis Pe394
P. sp. Pe411
P. pugnicaudex Pe412
P. peruviana Pe116
P. peruviana Pe208
P. peruviana Pe80
P. peruviana Pe82
P. peruviana Pe104
P. peruviana Pe108
P. peruviana Pe112
P. peruviana Pe438
P. peruviana Pe173
P. polycephala Pe443
P. sp. Pe421
P. sp. Pe422
P. gigantea Pe358
P. klopfensteinii Pe397
P. rupiseda Pe407
P. pinoi aff. Pe474
0.0
0.01
0.02
0.03
0.04
0.05
substitutions per site

Figure 5 (previous page). **Phylogram of the *trnK-matK-psbA* full dataset based on a Bayesian inference with relative substitution rates using the GTR+G model and posterior probabilities (PP) plotted above the branches. Clades are colored according to Figure 3.**

Multicopy

As described under ‘Structure and characterization of *agt1*’, approximately 90 % of all sequences did not require cloning to obtain distinct sequences. Only 10 % required an elaborated procedure. Putative pseudogenes, that showed e.g. frameshift mutations, were removed from the analysis. Multiple copies were detected in at least one accession from every clade except clade I and III. A resulting phylogeny, which contained all detected copies and which did not comprise the downstream part of intron III, revealed that the copies of eight accessions are monophyletic and thus do not influence the final analysis (Appendix 4). Thus, a cloned copy representing the particular accession could randomly be chosen for the main phylogeny. Copies of four accessions are not monophyletic. These copies cluster together with copies of other accessions of the respective species, indicating duplication during radiation of the respective species (*P. cerrateae* Pe417, *P. parvisagittata* Pe113, *P. schizandra* Pe24 and *P. wernerrauhii* Pe481). The only accessions for which the situation seems to be more complex are *P. bracteata* Pe28 and Pe32, as well as *P. cyclaminoides* Pe115 and *P. verruculosa* Pe414. Both cases show a similar pattern: copies of a single accession are not monophyletic, but are sister to their orthologous copy of other accessions. Thus, cloned sequences of such an accession are paralogs.

Discussion

Rapidly radiating genera and single copy nuclear genes

Rapidly radiating genera and species-rich groups have been, and remain, challenging targets for high resolution phylogenetic analysis with widely used chloroplast markers (e.g. Berry *et al.* 2005, Richardson *et al.* 2004, Kårehed *et al.* 2008). Recently, the advent of massively parallel sequencing decreased sequencing costs dramatically by a multiplexing approach, which allows gathering several plastid genomes at the time (Parks *et al.* 2009). This approach may become routine even for all species in large genera, and this will greatly improve phylogenetic resolution. However, it has to be borne in mind that maternal histories alone may be insufficient evidence. Nuclear ribosomal molecular markers are widely used markers at the species level, but depending on the group in question, their signal across deep branches of angiosperm phylogeny may be insufficient. Additionally, given their occurrence in large gene family arrays containing numerous paralogs, their utility has been questioned for species and population level analyses (Alvarez & Wendel 2003). Providing a nearly inexhaustible number of genes and gene families, the nucleus itself is a vast source for new molecular markers which are required to investigate numerous biological questions and to open up new prospects especially for studies focused on species level processes. In the present work a single copy nuclear gene that is shared between diverse angiosperms (Duarte *et al.* 2010) is applied to investigate its performance for resolving species level history for a rapidly radiating group. In this study, a collection of 13 of the genes best represented in plant EST datasets was used to generate a highly resolved phylogeny of 69 species of seed plants including most major gymnosperm and angiosperm lineages. Since there are giant genera in the realm of animals as well, an approach for identifying shared nSCG in a similar fashion that was applied by Duarte *et al.* (2010) would be highly beneficial.

Several exons of nSCG were identified to be very similar to both coding and non-coding chloroplast markers in terms of variability. They are functionally conserved in contrast to their introns, which show significantly increased substitution rates and numerous indels due to lack of functional selection, which results in an increased phylogenetic content. In this study, the *atg1* intronic regions diverged rapidly enough to resolve relationships on species level and below. The combination of introns and exons are highly applicable for resolving low-level relationships; if large enough intronic regions are available, these may in some cases permit resolution to the population level. Shaw (2007) concluded that non-coding chloroplast regions are the most useful for resolving relationships of closely related species. However, non-coding parts of all chloroplast markers employed in the present study are very similar in terms of variability compared to the coding sections of the

chloroplast genome on species level. This means that chloroplast introns and intergenic spacers are limited in resolving relationships on this very low taxonomic level (e.g. Richardson *et al.* 2004). In contrast to this, non-coding parts of nuclear genes show a three-fold increased variability compared to non-coding chloroplast markers. A deep sampling of *Peperomia* subgenus *Tildenia* showed significantly increased resolution and higher support values for the nuclear-derived tree compared to chloroplast trees. Depending on the taxonomic lineage, highly conserved coding parts of nSCG are useful for family level (Wiegman *et al.* 2009, Duarte *et al.* 2010), at least if only a handful of genes are applied. In contrast to LCNG (Sang 2002), shared nSCG (Duarte et al 2010) were selected to reflect a conserved copy number between disparate taxonomic groups. In the near future, many more coding regions of genes might be available for low cost in a multiplex approach allowing reconstruction of genus level, but likely not on species or population level.

For these reasons, shared nSCG are highly versatile and universal candidate markers for numerous biological evolutionary applications. The significantly higher concentration of PIC that can be detected compared to commonly used chloroplast markers is accompanied by less effort and lower sequencing costs once the primers and amplification conditions have been developed.

Gene tree versus species tree

Eukaryotic genomes and plant genomes in particular, have been subject to numerous events of whole genome duplication (WGD). There is evidence that all seed plants and angiosperms may have undergone ancestral WGDs, and there are at least three more recent polyploidy events detectable in the genome of *Arabidopsis* (Soltis & Soltis 2000, Cui *et al.* 2006, Jiao *et al.* 2011). Thus, any nuclear gene has been duplicated several times in its history resulting in gene families. Duplicated genes can take different fates subsequent such events: subfunctionalization, neofunctionalization or nonfunctionalization (Lynch & Conery 2000). Genes that commonly appear as conserved low copy have likely been through repeated rounds of duplication gene loss. There are various reasons for those duplicated copies to be nonfunctionalized and lost after polyploidization. In Duarte *et al.* (2010) it is suggested that both selection and random loss can account for these processes.

Since gene trees are not necessarily congruent to the species tree, several markers from different organellar genomes need to be applied (Nichols 2001). For gaining a species tree rather than gene trees, it is required to observe congruence across multiple unlinked loci. Since the chloroplast genes are a single linked group, it cannot be considered exclusively. Nuclear genes do evolve independently and thus, the nucleus provides a vast

repertory of unlinked markers. However, since there are thousands of genes in the nucleus, marker selection is challenging and the majority of nuclear genes occur in small to huge gene families. The conserved single copy nuclear marker applied in this paper and shared single copy genes in general do provide simplicity in their usage with a lab effort as low as chloroplast markers. As observed in the present and other studies (e.g. Albach *et al.*, 2010, Doust *et al.* 2007), different parts of the genome evolve differently and thus often may conflict in their signal. To process data like this, different software will be required to reconcile conflicting gene trees and to gain species trees (e.g. Page 1998). It is essential to use several unlinked markers for phylogenetic reconstruction to eliminate gene tree artifacts, which might be caused by hybridization, polyploidy, introgression or lineage sorting (Hughes 2006).

The *agt1* gene that is applied in this paper is one of the APVO SSCG set (Duarte *et al.* 2010). The constantly increasing number of sequenced plant genomes allows for identifying genes that are even more conserved single copy throughout all plants. Each genome that is added to this genome decreases the number of shared single copy genes. Planttribes 2.0 (http://fgp.huck.psu.edu/tribedb/10_genomes/index.pl?action=home) provides a publicly available tool that can be used for identifying such genes. If this approach is run with seven angiosperm genomes (*Arabidopsis*, *Populus*, *Vitis*, *Oryza*, *Sorghum*, *Medicago* and *Carica*), this number decreases to 223 genes and if three non-angiosperm plant genomes are added (*Selaginella*, *Physcomitrella*, and *Chlamydomonas*), 36 genes pass the criteria. However, this approach identifies genes that are very likely single copy throughout plants in general, but exceptions may occur in a particular lineage. In the case of *agt1*, beside the APVO species, it occurs in a single copy in *Sorghum bicolor* and *Carica papaya*, and in six copies in *Medicago truncatula*. In the three non-angiosperm plant genomes, two copies can be identified in *Selaginella moellendorfii*, three in *Chlamydomonas reinhardtii*, and five in *Physcomitrella patens*.

Conclusions

Considering all of the patterns observed for the *atg1* gene within this study (topology, variability and homoplasy), this nuclear singe copy gene is highly valuable for phylogenetics on a low taxonomic level such as species level and below. Beyond that, *atg1* was useful for identifying further evolutionary biological processes such as recent gene duplication. When combined with other nuclear genes duplications at many nSCGs would be indicative of recent polyploidization in a species or even in particular populations. In combination with uniparentally inherited loci, biparentally inherited nSCGs can also provide evidence for hybridization and potentially for organelle capture. Due to both a restricted range of variability and its uniparental inheritance, chloroplast molecular markers are insufficient without independent nuclear loci to address questions like this. In contrast, the nSCG applied in the present study provides regions with different quantitative levels of variability deriving from coding and non-coding parts of different length. While the percentage of PIC of the coding sections does not vary significantly among the chloroplast and nuclear markers, the non-coding sections of the nSCG in *Peperomia*, *atg1* is three times higher than even the most rapidly evolving chloroplast regions.

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Appendix

Appendix 1. **List of species used in this chapter including voucher information, field or garden origin as well as coordinates and altitude of the collection site.**

Taxon	Lab number	Field origin (country, state/department)	coordinates	altitude	Voucher (GENT herbarium)
P. andina Pino	Pe492	Peru, Ancash	09°31'37,2"S 77°32'08,4"W	3050 m	Mathieu & Symmank 2009-003
P. ayacuchoana Pino & Samain	Pe428*	Peru, Ayacucho	12°56'37,0"S 74°01'15,0"W	3190 m	Samain et al. 2009-040
P. basiradicans G.Mathieu	Pe4*	Mexico, Mexico	19°05'16"N 98°40'45.2"W	2170 m	Samain, Symmank & Mathieu 2007-009
P. bracteata Hill	Pe16*	Mexico, Mexico	17°17'10"N 99°28'20"W	750 m	Samain, Symmank & Mathieu 2007-013b
	Pe28*	Mexico, Michoacán	18°55'07.4"N 99°37'38.6"W	1960 m	Samain, Symmank & Mathieu 2007-052
	Pe31*	Mexico, Michoacán	19°29'57.6"N 101°49'47.4"W	2130 m	Samain, Symmank & Mathieu 2007-056
	Pe32*	Mexico, Michoacán	19°27'49.6"N 101°36'39.1"W	2260 m	Samain, Symmank & Mathieu 2007-057
	Pe33	Mexico, Michoacán	19°32'21"N 101°42'00.5"W	1990 m	Samain, Symmank & Mathieu 2007-058
P. cavispicata G.Mathieu	Pe14*	Mexico, Mexico	18°55'07.4"N 99°37'38.6"W	1960 m	Samain, Symmank & Mathieu 2007-026
P. cerrateae Pino & G. Mathieu	Pe111	Bolivia, Tarija	21°06'50,2"S 64°41'42,1" W	3000 m	Samain, Bates & Wanke 2008-081
	Pe171	Bolivia, La Paz	15°43'40.9"S 68°39'26,2"W	3200 m	Symmank, Mathieu & Sivila 2008-041
	Pe222*	Bolivia, Cochabamba	17°15'47,8"S 66°20'59,3"W	4080 m	Samain, Bates & Wanke 2008-146
	Pe417	Peru, Junín	12°16'55,4"S 75°42'06,4"W	4010 m	Samain et al. 2009-023
P. chutanka Pino	Pe436*	Peru, Lima	12°09'17"S 76°23'40"W	3320 m	Samain et al. 2009-076
P. cyclaminoides Hill	Pe110	Bolivia, Tarija	21°09'16,5"S 64°41'32,0"W	2940 m	Samain, Bates & Wanke 2008-080
	Pe114*	Bolivia, Tarija	21°27'20,8"S 64°52'39,6"W	3130 m	Samain, Bates & Wanke 2008-084
	Pe115	Bolivia, Tarija	21°29'52,7"S 64°54'11,3"W	3825 m	Samain, Bates & Wanke 2008-085
	Pe117	Bolivia, Chuquisaca	20°45'00,1"S 64°52'20,2"W	3130 m	Samain, Bates & Wanke 2008-087
	Pe219	Bolivia, Cochabamba	17°45'0,6"S 65°29'57,4"W	3580 m	Samain, Bates & Wanke 2008-130
P. dolabella Rauh & Kimnach	Pe392*	Peru, Cajamarca	07°10'34,1"S 78°31'53,8"W	3132 m	Mathieu, Symmank & Pino 2009-118
P. elatior G.Mathieu	Pe12	Mexico, Mexico	18°52'33.4"N 100°17'09"W	1500 m	Samain, Symmank & Mathieu 2007-035
P. exclamationis G.Mathieu	Pe25	Mexico, Michoacán	18°41'20.1"N 103°24'44.6"W	1260 m	Samain, Symmank & Mathieu 2007-045
P. gigantea G.Mathieu	Pe358	Peru, Cajamarca	07°09'25,5"S 78°50'30,4"W	1770 m	Mathieu, Symmank & Cieza 2009-158
P. jalcaensis Pino	Pe394	Peru, Cajamarca	07°11'19,8"S 78°34'32,3"W	3639 m	Mathieu, Symmank & Pino 2009-120
P. klopfensteinii Pino & Cieza	Pe397*	Peru, Cajamarca	06°39'32,3"S 78°44'01,4"W	2450 m	Mathieu, Symmank & Cieza 2009-130
P. ovatopeltata C.DC.	Pe42	Mexico, Morelos	18°59'10.7"N 99°06'07.7"W	2000 m	Samain, Symmank & Mathieu 2007-122
P. ovatopeltata C.DC. aff.	Pe41*	Mexico, Morelos	19°01'11.3"N 99°09'12"W	2260 m	Samain, Symmank & Mathieu 2007-124
	Pe43*	Mexico, Morelos	18°59'10.7"N 99°06'07.7"W	1725 m	Samain, Symmank & Mathieu 2007-120
P. parvifolia C.DC.	Pe420*	Peru, Huancavelica	12°20'40,8"S 74°56'13,0"W	4180 m	Samain et al. 2009-027
P. parvisagittata G.Mathieu & Pino	Pe113*	Bolivia, Tarija	21°11'14,6"S 64°46'18,9"W	3060 m	Samain, Bates & Wanke 2008-083

	Pe352	Peru, Ancash	10°09'31,4"S 77°21'13,3"W	3480 m	Mathieu & Symmank 2009-020
P. peruviana Dahlst.	Pe80	Bolivia, Cochabamba	17°24'03,3"S 65°48'24,9"W	3180 m	Samain, Bates & Wanke 2008-015
	Pe82	Bolivia, Cochabamba	17°42'46,2"S 66°33'14,2"W	3930 m	Samain, Bates & Wanke 2008-013
	Pe104	Bolivia, Cochabamba	17°27'25,6"S 65°44'10,7"W	3185 m	Samain, Bates & Wanke 2008-024
	Pe108*	Bolivia, Tarija	21°26'29,8"S 64°23'08,8"W	2335 m	Samain, Bates & Wanke 2008-075
	Pe112	Bolivia, Tarija	21°11'14,6"S 64°46'18,9"W	3060 m	Samain, Bates & Wanke 2008-082
	Pe116	Bolivia, Chuquisaca	20°46'06,4"S 64°52'20,2"W	2940 m	Samain, Bates & Wanke 2008-086
	Pe173	Bolivia, La Paz	15°27'51.4"S 69°05'24,1"W	3925 m	Symmank, Mathieu & Sivila 2008-045
	Pe208	Bolivia, Chuquisaca	19°04'52,7"S 64°49'23,2"W	2755 m	Samain, Bates & Wanke 2008-092
	Pe438*	Peru, Puno	15°39'41,3"S 70°28'13,0"W	3950 m	Samain et al. 2009-081
P. polycephala Trel.	Pe443	Peru, Cuzco	13°34'39,1"S 71°42'41,9"W	3130 m	Samain et al. 2009-105
P. pinoi G.Mathieu aff.	Pe474*	Peru, Huánuco	12°22'02,4"S 74°48'20,7"W	3560 m	Samain et al. 2009-230
P. pseudoverruculosa G.Mathieu	Pe437	Peru, Arequipa	15°36'41,9"S 15°36'41,9"W	3680 m	Samain et al. 2009-077
P. pugnicaudex Pino	Pe412*	Peru, Lima	11°44"24,4"S 76°16'48,7"W	3430 m	Samain et al. 2009-007
P. putlaensis G.Mathieu	Pe9*	Mexico, Oaxaca	17°07'51.1"N 97°52'20.2"W	1315 m	Samain, Symmank & Mathieu 2007-105
	Pe48	Mexico, Oaxaca	17°11'57.6"N 97°53'17.2"W	2015 m	Samain, Symmank & Mathieu 2007-104
P. questionis G.Mathieu	Pe15	Mexico, Guerrero	17°29'00.2"N 99°25'45.2"W	1060 m	Samain, Symmank & Mathieu 2007-014
P. radiatinervosa G.Mathieu	Pe61	Bolivia, Cochabamba	17°33'35,6"S 65°20'25,4"W	3030 m	Samain, Bates & Wanke 2008-028
	Pe62	Bolivia, Cochabamba	17°44'38,2"S 64°58'33,4"W	3015 m	Samain, Bates & Wanke 2008-029
	Pe81	Bolivia, Cochabamba	17°23'06,0"S 66°08'07,3"W	2830 m	Samain, Bates & Wanke 2008-014
	Pe211	Bolivia, Chuquisaca	18°41'36,3"S 64°41'37,3"W	2670 m	Samain, Bates & Wanke 2008-105a
P. rupiseda C.DC.	Pe407*	Peru, Lima	11º26'51"N 76º36'57"W	2750 m	Samain et al. 2009-001
P. schizandra Trel.	Pe21	Mexico, Jalisco	20°49'36.4"N 103°20'47.2"W	1250 m	Samain, Symmank & Mathieu 2007-038
	Pe24	Mexico, Michoacán	18°95'32.7"N 103°03'36.3"W	780 m	Samain, Symmank & Mathieu 2007-043
P. sp.	Pe2*	Mexico, Guerrero	18°37'12.3"N 99°38'14.9"W	2330 m	Samain, Symmank & Mathieu 2007-023
P. sp.	Pe411*	Peru, Lima	11°42'15,6"S 76°16'04,9"W	3790 m	Samain et al. 2009-005
P. sp.	Pe421	Peru, Huánuco	12°22'02,4S 74°48'20,7"W	3560 m	Samain et al. 2009-028
P. sp.	Pe422*	Peru, Huánuco	12°22'02,4S 74°48'20,7"W	3560 m	Samain et al. 2009-029
P. sp.	Pe481	Peru, Huánuco	10°14'26,5"S 76°09'13,2"W	2700 m	Samain et al. 2009-213
P. verruculosa Dahlst. ex Hill	Pe105	Bolivia, Cochabamba	12°20'40,8S 74°56'13,0"W	4180 m	Samain, Bates & Wanke 2008-027
	Pe414	Peru, Junín	11°27'07"S 75°56'56,6"W	3810 m	Samain et al. 2009-009
	Pe442*	Peru, Puno	14°36'23,0"S 14°36'23,0"W	4060 m	Samain et al. 2009-101
P. wernerrauhii Pino & Samain	Pe482	Peru, Huánuco	09°53'36,8"S 76°09'03,2"W	2470 m	Samain et al. 2009-214

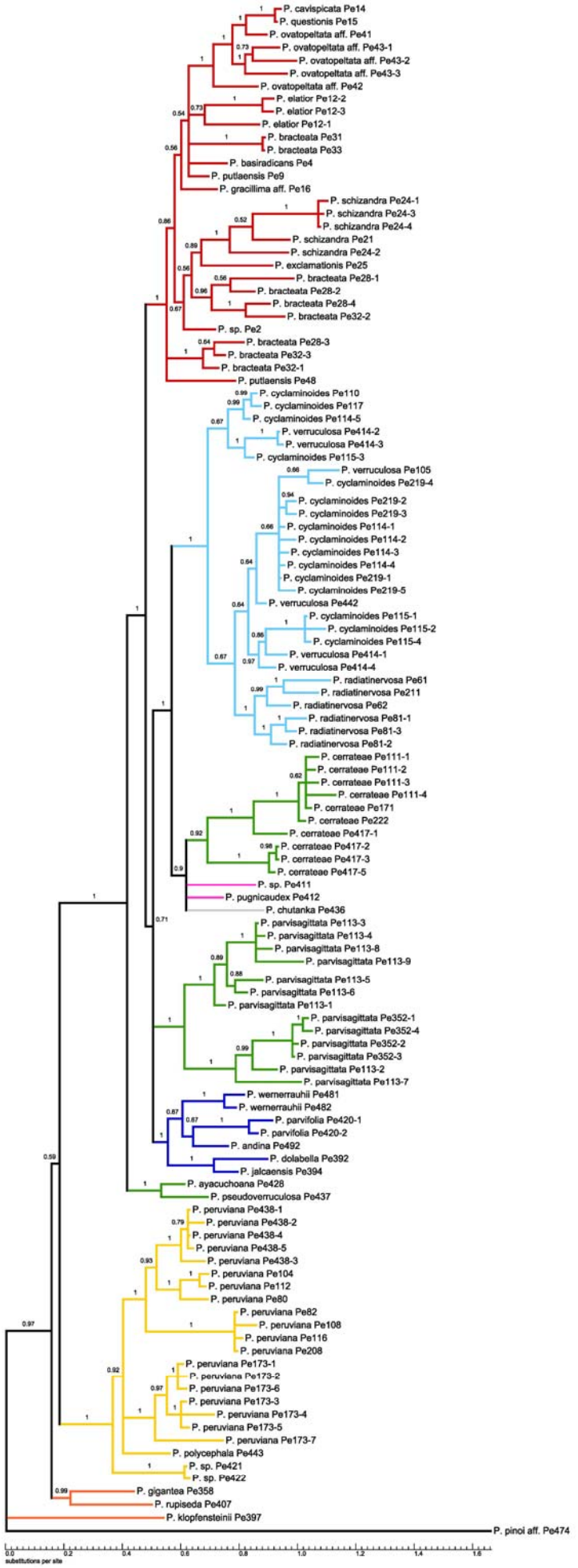
* sampling for reduced datasets

Appendix 2. Primers used in this study.

Region	Primer name	Direction	Sequence	Taxonomic group	Design
agt1	AUAP	reverse	GGCCACGCGTCGACTAGTAC	universal	Invitrogen 3'RACE System for Rapid Amplification of cDNA Ends
	M13-F	forward	GTAAAACGACGGCCAG	universal	Invitrogen TOPO TA cloning Instruction manual (2004)
	M13-R	reverse	CAGGAAACAGCTATGAC	universal	Invitrogen TOPO TA cloning Instruction manual (2004)
	Pe-4474-550F	forward	GAAACAATGAGGACTACCGCT	*Tildenia* + outgroup	this study
	Pe-4474-ExIIa-F1	forward	CTGACCAAAACMCTSCTTGA	outgroup	this study
	Pe-4474-ExIIb-F1	forward	ACCGAACCATYTCATTCTTGATT	*Tildenia* + outgroup	this study
	Pe-4474-ExIIb-R1	reverse	ATCTTTCTWACTGTTGCYAA	*Tildenia*	this study
	Pe-4474-ExIIb-R2	reverse	GCAAGTTTTGAWGMCAGAAT	*Tildenia*	this study
	Pe-4474-ExIII-F1	forward	TTCTTTGAYTGGAATGAYTACTT	*Tildenia*	this study
	Pe-4474-ExIII-F2	forward	AGGGTTTTCTTTGAYTGGAATGA	outgroup	this study
	Pe-4474-ExIII-R1	reverse	TTACCTTGTGGCTTTGCCCAA	*Tildenia*	this study
	Pe-4474-ExIII-R3	reverse	CGAGYCCTTCCTCAAAGATRA	*Tildenia* + outgroup	this study
	Pe-4474-ExIII-R4	reverse	ATGAYACTCCATCCACAAGAA	outgroup	this study
	Pe-4474-ExV-R3	reverse	GGATYAATGGWGTWGTGTTCTG	*Tildenia*	this study
	Pe-4474-ExV-R5	reverse	TAAGAACCATCTCCACTCCACT	*Tildenia* + outgroup	this study
ndhF-rpl32-trnL	Pe-ndhF2	forward	GGAGTGGAATAAAAGGTATGAT	*Tildenia* + outgroup	this study
	Pe-rpL32-F	forward	ATGRCGGTTCCAAAGAAACG	*Tildenia* + outgroup	this study
	Pe-rpL32-R	reverse	ACTTTTWGAATGTCCAGTAGAAAT	*Tildenia* + outgroup	this study
	trnL-(UAG)	reverse	CTGCTTCCTAAGAGCAGCGT	*Tildenia* + outgroup	Shaw et al. (2005,2007)
psbA-trnH	Pe-trnH-R	reverse	RATTCACAATCCACTGCCTTGAT	*Tildenia* + outgroup	this study
	Pe-psbA-F	forward	AATGCACACAACTTCCCTCTA	*Tildenia* + outgroup	this study

Appendix 3. Shimodaira–Hasegawa (SH) test results of different topology/dataset combinations. P-values ≤ 0,(asterisk) indicate a significant rejection of the respective combination. Alternative hypothesis manually changed from the obtained topologies of the respective analyses are given in Newik format.

Topologies		Dataset			
		trnK-matK-psbA	***agt1***	***ndhF-rpl32-trnL***	***trnH-psbA***
full sampling	***trnK-matK-psbA***	best	0,000*		
	agt1	0,000*	best		
small sampling	***trnK-matK-psbA***	best	0,004*	0,474	0,518
	agt1	0,011*	best	0,070	0,532
	ndhF-rpl32-trnL	0,220	0,005*	best	best
	trnH-psbA	0,000*	0,000*	0,000*	0,115
alternative hypothesis (genetic marker)					
1 (*agt1*) (Pe474,Pe397,(((((((Pe2,(Pe4,Pe9,((Pe14,Pe41),Pe43),Pe31),Pe16),(Pe28,Pe32)),Pe436),Pe113,(Pe392,Pe420)),((Pe114,Pe442),((Pe222,Pe412),Pe411))),Pe428),(((Pe108,Pe438),Pe422),Pe407)))		0,016*			
2 (*agt1*) (Pe474,Pe397,((((Pe108,Pe438),Pe422),Pe407),((((Pe2,(Pe4,Pe9,((Pe14,Pe41),Pe43),Pe31),Pe16),(Pe28,Pe32)),(Pe392,Pe420)),(((Pe114,Pe442),(Pe222,Pe113,Pe428)),((Pe411,Pe412),Pe436)))))		0,037*			
3 (*agt1*) (Pe474,Pe397,(((((((Pe2,(Pe4,Pe9,((Pe14,Pe41),Pe43),Pe31),Pe16),(Pe28,Pe32)),Pe113,(Pe392,Pe420)),((Pe114,Pe442),(Pe436,Pe222))),Pe428),(Pe412,Pe411)),(((Pe108,Pe438),Pe422),Pe407)))		0,020*			
4 (*agt1*) (Pe474,Pe397,((((Pe108,Pe438),Pe422),Pe407),((((Pe2,(Pe4,Pe9,((Pe14,Pe41),Pe43),Pe31),Pe16),(Pe28,Pe32)),(Pe392,Pe420)),((Pe114,Pe442),((((Pe222,Pe428,Pe113),Pe412),Pe411),Pe436))))));):0.003474):0.002743):0.003323):0.015545)		0,024*			
5 (*agt1*) (Pe474,Pe397,((((Pe108,Pe438),Pe422),Pe407),((((Pe2,(Pe4,Pe9,((Pe14,Pe41),Pe43),Pe31),Pe16),(Pe28,Pe32)),(Pe392,Pe420),(Pe222,Pe428,Pe113)),((Pe114,Pe442),((Pe411,Pe412),Pe436)))))		0,030*			
6 (*agt1*) (Pe474,Pe397,((((Pe108,Pe438),Pe422),Pe407),(((((Pe2,(Pe4,Pe9,((Pe14,affPe41),Pe43),Pe31),Pe16),(Pe28,Pe32)),(Pe392,Pe420)),((Pe114,Pe442),((Pe411,Pe412),Pe436))),(Pe222,Pe428,Pe113))))		0,036*			
7 (*agt1*) (Pe474,Pe397,((((Pe108,Pe438),Pe422),Pe407),(((((Pe2,(Pe4,Pe9,((Pe14,Pe41),Pe43),Pe31),Pe16),(Pe28,Pe32)),(Pe392,Pe420)),((Pe114,Pe442),((Pe411,Pe412),Pe436))),(Pe222,Pe428,Pe113))))		0,030*			
8 (*trnK-matK-psbA*) (Pe474,((((((((((Pe2,Pe9),(Pe41,Pe43)),Pe14),(Pe4,((Pe28,Pe31),Pe32))),Pe16),Pe436),(Pe392,Pe420)),(((Pe113,Pe222),Pe428),(Pe114,Pe442))),(Pe411,Pe412)),((Pe108,Pe438),Pe422)),(Pe397,Pe407))			0,004*		
9 (*trnK-matK-psbA*) (Pe474,(((((Pe428,Pe113),(Pe114,Pe442)),(Pe392,Pe420),(((((Pe2,Pe9),(Pe41,Pe43)),Pe14),(Pe4,((Pe28,Pe31),Pe32))),Pe16)),((Pe411,(Pe412,Pe222)),Pe436)),((Pe108,Pe438),Pe422)),(Pe397,Pe407))			0,023*		
10 (*trnK-matK-psbA*) (Pe474,(((Pe108,Pe438),Pe422),((((Pe428,Pe113),((Pe411,(Pe412,Pe222)),Pe436)),(Pe114,Pe442)),(Pe392,Pe420),(((((Pe2,Pe9),(Pe41,Pe43)),Pe14),(Pe4,((Pe28,Pe31),Pe32))),Pe16))),(Pe397,Pe407))			0,017*		



Appendix 4.

Phylogram from the Bayesian inference of the *agt1* full data set containing all cloned sequences. Relative substitution rates calculated using the GTR+G+I model. Posterior probabilities (PP) plotted above the branches. Clades are colored according to Figure 3. The applied data set comprises part I and part II of the nuclear gene. Different clones of one accession are consecutively numbered the end of branch label.

REFERENCES

Alvarez, I., Costa, A. & Feliner, G.N. (2008) Selecting Single-Copy Nuclear Genes for Plant Phylogenetics: A Preliminary Analysis for the Senecioneae (Asteraceae). *Journal of Molecular Evolution*, **66**, 276-291.

Albach, D.C. & Meudt, H.M. (2010) Phylogeny of Veronica in the Southern and Northern Hemispheres based on plastid, nuclear ribosomal and nuclear low-copy DNA. *Molecular Phylogenetics and Evolution*, **54**, 457-471.

Alvarez, I. & Wendel, J.F.(2003) Ribosomal ITS sequences and plant phylogenetic inference. *Molecular Phylogenetics and Evolution*, **29**, 417-434.

Angiosperm Phylogeny Group (2009) An update of the Angiosperm Phylogeny Group classification for the orders and families of flowering plants: APG III. *Botanical Journal of the Linnean Society*, **161**,105-121.

Angiosperm Phylogeny Website [http://www.mobot.org/mobot/research/apweb/].

Bailey, C.D. & Doyle, J.J. (1999) Potential Phylogenetic Utility of the Low-Copy Nuclear Gene pistillata in Dicotyledonous Plants: Comparison to nrDNA ITS and trnL Intron in Sphaerocardamum and Other Brassicaceae. *Molecular Phylogenetics and Evolution*, **1**, 20-30.

Bailey, C.D., Carr, T., Harris, S. & Hughes, C. (2003) Characterization of angiosperm nrDNA polymorphim, paralogy, and pseudogenes. *Molecular Phylogenetics and Evolution*, **29**, 435-455.

Barkman, T.J., McNeal, J.R., Lim, S-H., Coat, G., Croom, H.B., Young, N.D. & dePamphilis, C.W. (2007) Mitochondrial DNA suggests at least 11 origins of parasitism in angiosperms and reveals genomic chimerism in parasitic plants. *BMC Evolutionary Biology*, **7**,248.

Barraclough, T., Nee, S. (2001) Phylogenetics and speciation. *Trends in Ecology and Evolution*, **16**, 391-399.

Barrier, E., Velasquillo, L., Chavez, M. & Gaulon, R. (1998) Neotectonic evolution of the Isthmus of Tehuantepec (Southeastern Mexico). *Tectonophysics*, **287**, 7-96.

Barthlott, W., Mutke, J., Rafiqpoor, M.D., Kier, G. & Kreft, H. (2005) Global centres of vascular plant diversity. *Nova Acta Leopoldina*, **92**, 61-83.

Bell, C.D. & Donoghue, M.J. (2005) Phylogeny and biogeography of Valerianaceae (Dipsacales) with special reference to the South American valerians. *Organisms Diversity & Evolution*, **5**. 147-159.

Bell, C.D., Soltis, D.E. & Soltis, P.S. (2010) The age and diversification of the angiosperms re-revisited. *American Journal of Botany*, **97,** 1296-1303.

Bermingham, E., & Martin, A.P. (1998) Comparative mtDNA phylogeography of neotropical freshwater fishes: testing shared history to infer the evolutionary landscape of lower Central America. *Molecular Ecology,* **7**, 499-517.

Berry, P.E, Hipp, A.L., Wurdack, K.J., Van Ee, B. & Riina, R. (2005) Molecular phylogenetics of the giant genus Croton and tribe Crotoneae (Euphorbiaceae sensu stricto) using ITS and trnL-trnF DNA sequence data. *American Journal of Botany*, **92**, 1520–1534.

Borsch, T., Hilu, K.W., Quandt, D., Wilde, V., Neinhuis, C. & Barthlott, W., (2003) Noncoding plastid *trnT-trnF* sequences reveal a well resolved phylogeny of basal angiosperms. *Journal of Evolutionary Biology*. **16**, 558-576.

Bradley, U. (2002) Biogeography and speciation of the genus Peperomia Ruiz and Pavon in Eastern Polynesia. PhD thesis. University of Dublin, Trinity College.

Britton, T., Anderson, C.L., Jacquet, D., Lundqvist, S. & Bremer, K. (2007) Estimating divergence times in large phylogenetic trees. *Systamatic Biology*, **56**, 741-752.

Bruyns, P.V., Mapaya, J.R. & Hedderson T. (2006) A new subgeneric classification for Euphorbia (Euphorbiaceae) in southern Africa based on ITS and psbA-trnH sequence data. *Taxon***, 55**, 397–420.

Burger, W.C. (1971). Flora Costaricensis - Family 41 Piperaceae. *Fieldiana Botany*, **35**, 5–79.

Burnham, R.J. & Graham, A. (1999) The history of Neotropical vegetation: new developments and status. *Missouri Botanical Garden,* **86**, 546-589.

Bush, M.B. (1994) Amazonian speciation: a necessarily complex model. *Journal of Biogeography*, **21**, 5-17.

Chapman, B.A., Bowers, J.E., Feltus, F.A. & Paterson, A.H. (2006) Buffering of crucial functions by paleologous duplicated genes may contribute cyclicality to angiosperm genome duplication. *Proceedings of the National Academy of Sciences*, **103**, 2730-2735.

Childs, K.L., Hamilton, J.P., Zhu, W., Ly, E., Cheung, F., Wu, H., Rabinowicz, P.D., Town, C.D., Buell, C.R., Chan, A.P (2007) The TIGR Plant Transcript Assemblies database. *Nucleic Acids Research*, **35**, D846-D851.

Coates, A.G. & Obando, J.A. (1996) The geologic evolution of the Central American Isthmus. *Evolution and environment in tropical America* (ed. by J.B.C. Jackson, A.F. Budd and A.G. Coates), pp. 21-56. University of Chicago Press, Chicago.

Coates, A.G., Aubry, M-P., Berggren, W.A. & Collins, L.S. (2003) Early Neogene history of the Central American arc from Bocas del Toro, western Panama. *Geological Society of America Bulletin*, **115**, 271-287.

Cody, S., Richardson, J.E., Rull, V., Ellis, C., & Pennington, R.T. (2010) The great American biotic interchange revisited. *Ecography*, **33**, 326-332.

Colless, D.H. (1980) Congruence between morphometric and allozyme data for Menidia species: a reappraisal. *Systematic Zoology*, **29**, 288-299.

Collins, A.C. & Dubach, J.M. (2000) Biogeographic and ecological forces responsible for speciation in *Ateles*. *International Journal of Primatology*, **21**, 421-444.

Cosacov, A., Sérsic, A.N., Sosa, V., De-Nova, J.A., Nylander, S. & Cocucci, A.A. (2009) New insights into the phylogenetic relationships, character evolution, and phytogeographic patterns of *Calceolaria* (Calceolariaceae). *American Journal of Botany*, **96**, 2240-2255.

Cui, L., Wall, P.K., Leebens-Mack, J.H., Lindsay, B.G., Soltis, D.E., Doyle, J.J., Soltis, P.S., Carlson, J.E., Arumuganathan, K., Barakat, A., Albert, V.A., Ma, H. & dePamphilis, C.W. (2006) Widespread genome duplications throughout the history of flowering plants. *Genome Research*, **16**, 738-749.

Dahlstedt, H. (1900) Studien über süd- und central-amerikanische Peperomien mit besonderer Berücksichtigung der brasilianischen Sippen. *Kungliga Svenska Vetenskapsakademiens Handlingar*, **33**, 1-218.

Doust, A.N., Penly, A.M., Jacobs, S.W.L. & Kellogg, E.A. (2007) Congruence, Conflict, and Polyploidization Shown by Nuclear and Chloroplast Markers in the Monophyletic ‘‘Bristle Clade’’ (Paniceae, Panicoideae, Poaceae). *Systematic Botany*, **32**,531-544.

Doyle, J.J., Doyle, J.L., Rauscher, J.T., Brown, A.H.D. (2004) Diploid and polyploid reticulate evolution throughout the history of the perennial soybeans (*Glycine* subgenus *Glycine*). *New Phytologist*, **161**,121-132.

dePamphilis, C.W. & Palmer, J.D. (1989) Evolution and Function of Plastid DNA: A Review With Special Reference to Nonphotosynthetic Plants. Physiology, biochemistry, and genetics of nongreen plastids (ed. By C.D. Boyer, J.C. Shannon and R.C. Hardison). *The American Society of Plant Physiologists*, **1989**,182-202.

Drummond, A.J. & Rambaut, A. (2007) BEAST: Bayesian evolutionary analysis by sampling trees. *BMC Evolutionary Biology,* **7**, 214.

Drummond, A.J, Ho, S.Y.W, Phillips, M.J. & Rambaut, A. (2006) Relaxed phylogenetics and dating with confidence. *PLOS Biology*, **4**, 699-710.

Duarte, J.M., Wall, K.P., Edger, P.P., Landherr, L.L., Ma, H., Pires, J.C., Leebens-Mack, J. & dePamphilis, C.W. (2010) Identification of shared single copy nuclear genes in *Arabidopsis*, *Populus*, *Vitis* and *Oryza* and their phylogenetic utility across various taxonomic levels. *BMC Evolutionary Biology*, **10**, 61.

Duque-Caro, H., (1990) Neogene stratigraphy, paleoceanography and paleobiogeography in northwest South America and the evolution of the Panama Seaway. Palaeogeography, Paleoclimatology, *Palaeoecology*, **77**, 203-234.

Ferrusquia-Villafranca, I. (1993) Geology of Mexico: A Synopsis. *Biological Diversity of Mexico. Origins and Distribution* (ed. by T.P. Ramamoorthy, R. Bye, A. Lot and J. Fa), pp. 3-107. Oxford University Press, New York.

Frodin, D.G. (2004) History and concepts of big plant genera. *Taxon*, **53**, 753-776.

Fulton, T.M., Van der Hoeven, R., Eannetta, N.T. & Tanksley, S.D. (2002) Identification, analysis, and utilization of conserved ortholog set markers for comparative genomics in higher plants. *Plant Cell*, **14**, 1457-1467.

Fulton, T.M., Van der Hoeven, R., Eannetta, N.T. & Tanksley, S.D. (2002) Identification, analysis, and utilization of conserved ortholog set markers for comparative genomics in higher plants. *Plant Cell*, **14**, 1457-1467.

Garzione, C.N. (2008) Surface uplift of Tibet and Cenozoic global cooling. *Geology*, **36**, 1003-1004.

Gentry, A.H. (1982) Neotropical floristic diversity: phytogeographical connections between Central and South America. Pleistocene climatic fluctuations, or an accident of the Andean orogeny? *Annals of the Missouri Botanical Garden*, **69**, 557-593.

Glor, R.E., Vitt, L.J & Larson, A. (2001) A molecular phylo-genetic analysis of diversification in Amazonian Anolis lizards. *Molecular Ecology* ,**10**, 2661-8.

Good-Avila, S. V., Souza, V., Gaut, B.S. & Eguiarte L.E. (2006) Timing and rate of speciation in *Agave* (Agavaceae). *Proceedings of the National Academy of Sciences*, **103**, 9124-9129.

Graham, A. (2009) The Andes: a geological overview from a biological perspective. *Annals of the Missouri Botanical Garden*, **96**, 371-385.

Gregory-Wodzicki, K.M. (2000) Uplift history of the Central and Northern Andes: A review. *Geological Society of America Bulletin*, **112**, 1091-1105.

Haber, W.A. (2000) Plants and vegetation. *Monteverde: ecology and conservation of a tropical cloud forest* (ed. by N.M. Nadkarni and N.T. Wheelwright), pp. 39–94. Oxford University Press, New York.

Haffer, J. (1969) Speciation in Amazonian forest birds. *Science*, **165**, 131-137.

Haffer, J. (2008) Hypotheses to explain the origin of species in Amazonia. *Brazilian Journal of Biologie*, **68**, 917-947.

Haq, B.U., Hardenbol, J. & Vail, P.R. (1987) Chronology of fluctuating sea levels since the Triassic. *Science*, **235**, 1156-1166.

Hartley, A.J. (2003) Andean uplift and climate change. *Journal of the Geological Society*, **160**, 7-10.

Hill, A.W. (1906) The morphology and seedling structure of the geophilous species of *Peperomia*, together with some views on the origin of monocotyledons. *Annals of Botany*, **20**, 395-427.

Hill, A.W. (1907) A revision of the geophilous species of *Peperomia* with some additional notes on their morphology and seedling structure. *Annals of Botany*, **21**, 141-160.

Ho, S.Y.W. (2007) Calibrating molecular estimates of substitution rates and divergence times in birds. Journal of Avian Biology, 38, 409-414.

Hooghiemstra, H. & Van der Hammen, T. (2004) Quaternary ice-age dynamics in the Colombian Andes: developing an understanding of our legacy. *Philosophical Transactions of the Royal Society of London Series B, Biological Sciences,* **359**, 173-181.

Hoorn, C., Guerrero, J., Sarmiento, G.A. & Lorente, M.A. (1995) Andean tectonics as a cause of changing drainage patterns in Miocene northern South America. *Geology*, **23**, 237–240.

Horn, S.P., Sanford, R.L., Dilcher, D., Lott, T.A., Renne, P.R., Wiemann, M.C., Cozadd, D. & Vargas, O. (2003) Pleistocene plant fossils in and near La Selva Biological Station, Costa Rica. *Biotropica*, **35**, 434-441.

Huelsenbeck, J.P. & Ronquist, F. (2001) MRBAYES: Bayesian inference of phylogenetic trees. *Bioinformatics*, **17**, 754-755.

Hughes, C., & Eastwood, R. (2006) Island radiation on a continental scale: exceptional rates of plant diversification after uplift of the Andes. *Proceedings of the National Academy of Sciences USA*, **103**, 10334-10339.

Hughes, C.E., Eastwood, R.J., Donovan & Bailey, C (2006) Review. From famine to feast? Selecting nuclear DNA sequence loci for plant species-level phylogeny reconstruction. *Philosophical Transactions of the Royal Society of London Series B, Biological Sciences*, **361**, 211-225.

Hungerbühler, D., Steinmann, M., Winkler, W., Seward, D., Eguez, A., Peterson, D.E., Helg, U. & Hammer, C., (2002) Neogene stratigraphy and Andean geodynamics of southern Ecuador. *Earth-Science Reviews,* **57**, 75-124.

Iturralde-Vinent, M.A. & MacPhee, R.D.E. (1999) Paleogeography of the Caribbean region: implications for Cenozoic biogeography. *Bulletin of the American Museum of Natural History*, **238**, 1-95.

Jansen, R.K., Cai, Z., Raubeson, L.A., Daniell, H., dePamphilis, C.W., Leebens-Mack, J., Müller, K.F., Guisinger-Bellian, M., Haberle, R.C., Hansen, A.K., Chumley, T.W., Lee, S., Peery, R., McNeal, J.R., Kuehl, J.V. & Boore, J.L. (2007) Analysis of 81 genes from 64 plastid genomes resolves relationships in angiosperms and identifies genome-scale evolutionary patterns. *Proceedings of the National Academy of Sciences*, **104**,19369-19374.

Jaramillo, M.A., Callejas, R., Davidson, C., Smith, J.F., Stevens, A.C. & Tepe E.J. (2008) A Phylogeny of the Tropical Genus Piper Using ITS and the Chloroplast Intron psbJ–petA. *Systematic Botany*, **33**, 647–660.

Jiao, Y., Wickett, N.J., Ayyampalayam, S., Chanderbali, A., Landherr, L., Ralph, P.E., Tomsho, L.P., Hu, Y., Liang, H., Soltis, P.S., Soltis, D.E., Clifton, S.W., Schlarbaum, S.E., Schuster, S.C., Ma, H., Leebens-Mack, J. & dePamphilis CW (2011) Ancestral polyploidy in seed plants and angiosperms. *Nature*, doi:10.1038/nature09916 Letter.

Kårehed, J., Groeninckx, I., Dessein, S., Motley, T.J. & Bremer, B. (2008) The phylogenetic utility of chloroplast and nuclear DNA markers and the phylogeny of the Rubiaceae tribe Spermacoceae. *Molecular Phylogenetics and Evolution*, **49**, 843-866.

Keigwin, L.D. (1978) Pliocene closing of the Isthmus of Panama based on biostratigraphic evidence from nearby Pacific and Caribbean sea cores. *Geology*, **6**, 630-634.

Keller, G. & Barron, J.A., (1983) Paleoceanographic implications of Miocene deep-sea hiatuses. *Geological Society of America Bulletin*, **94**, 590-613.

Kim, S., Sultan, S.E. & Donoghue, M.J. (2008) Allopolyploid speciation in Persicaria (Polygonaceae): Insights from a low-copy nuclear region. *Proceedings of the National Academy of Sciences*, **105**,12370-12375.

Knapp, S. (2002) Assessing patterns of plant endemism in Neotropical uplands. *The Botanical Review*, **68**, 22-37.

Kodandaramaiah, U. (2010) Use of dispersal-vicariance analysis in biogeography - a critique. *Journal of Biogeography*, **37**, 3-11.

Koonin, E.V. (2005) Orthologs, Paralogs, and Evolutionary Genomics. *Annual Review of Genetics*, **39**, 309-38.

Kreft, H. & Jetz, W. (2007) Global patterns and determinants of vascular plant diversity. *Proceedings of the National Academy of Sciences USA*, **104**, 5925-5930.

Krzywinski, J., Wilkerson, R.C. & Besansky, N.J. (2001) Toward understanding Anophelinae (Diptera, Culicidae) phylogeny: insights from nuclear single-copy genes and weight of evidence. *Systematic Biology*, **50**, 540-556.

Lavin, M., Schrire, B.P., Lewis, G., Pennington, R.T., Delgado−Salinas, A., Thulin, M., Hughes, C.E., Matos, A.B. & Wojciechowski, M.F. (2004) Metacommunity process rather than continental tectonic history better explains geographically structured phylogenies in legumes. *Philosophical Transactions of the Royal Society of London Series B, Biological Sciences*, **359**, 1509-1522.

Liang, H. & Hilu, K.W. (1996) Application of the *matK* gene sequences to grass systematics. *Canadian Journal of Botany*, **74**, 125-134.

Liepman, A.H. & Olsen, L.J. (2003) Alanine aminotransferase homologs catalyze the glutamate:glyoxylate aminotransferase reaction in peroxisomes of Arabidopsis. *Plant Physiology*, **131**, 215-227.

Lovejoy, N.R., Albert, J.S. & Crampton, W.G.R. (2006) Miocene marine incursions and marine/freshwater transitions. *Journal of South American Earth Sciences*, **21**, 5-13.

Lynch, M. & Conery, J.S. (2000) The evolutionary fate and consequences of duplicate genes. *Science*, **290**, 1151-1155.

MacPhail, M.K., Partridge, A.D. & Truswell, E.M. (1999) Fossil pollen records of the problematical primitive angiosperm family Lactoridaceae in Australia. *Plant Systematics and Evolution*, **214**, 199-210.

Maddison, D.R., Schulz, K.S. & Maddison, W.P. (2007) The Tree of Life Web Project. *Zootaxa*, **1668**, 19-40.

Magallón, S. (2009) Flowering plants (Magnoliophyta). *The timetree of life* (ed. by S.B. Hedges and S. Kumar). pp. 161-165. Oxford University Press, New York.

Magallón, S. (2010) Using Fossils to Break Long Branches in Molecular Dating: A Comparison of Relaxed Clocks Applied to the Origin of Angiosperms. *Systematic Biology*, **59**, 384-399.

Magallón, S. & Sanderson, M.J. (2001) Absolute diversification rates in angiosperm clades. *Evolution*, **55**, 1762-1780.

Marshall, C.J. & Liebherr, J.K. (2000) Cladistic biogeography of the Mexican transition zone. *Journal of Biogeography*, **27**, 203-216.

Mathieu, G. (2007) Compendium of herbarium names in the genus Peperomia (Piperaceae). Nautilus Academic Books, Zelzate.

Mathieu, G. (2009) Proposal to conserve the name *Tildenia mexicana* (*Peperomia mexicana*) against *Peperomia galeottiana* (Piperaceae). *Taxon*, **58**, 1374-1375.

Mathieu G., Callejas R. (2006) New synonymies in the genus *Peperomia* Ruiz & Pav. (Piperaceae) - an annotated checklist. *Candollea*, **61**, 331-363.

Mathieu, G., Symmank, L., Callejas, R., Wanke S., Neinhuis, C., Goetghebeur P. & Samain, M.S. (in press) New geophytic *Peperomia* (Piperaceae) species from Mexico, Belize and Costa Rica. *Revista Mexicana de Biodiversidad*, **82**, in press.

McLoughlin, S. (2001) The breakup history of Gondwana and its impact on pre-Cenozoic floristic provincialism. *Australian Journal of Botany*, **49**, 271-300.

McNeill, J., Barrie, F.R., Burdet, H.M., Demoulin, V., Hawksworth, D.L., Marhold, K., Nicolson, D.H., Prado, J., Silva, P.C., Skog, J.E., Wiersema, J.A. & Turland, N.J. (2006) International Code of Botanical Nomenclature (Vienna Code) Adopted by the Seventeenth International Botanical Congress, Vienna, Austria, July 2005. Königstein, Koeltz Scientific Books. [Regnum Veg. 146].

Meyen, F.J.F. (1834) Reise um die Erde ausgefürt auf dem Königlisch Seehandlungs-Schiffe Prinzess Louise in den Jahren 1830, 1831 und 1832. Vol.1., Sander'schen Buchhandlung, Berlin.

Miquel, F.A.W. (1843a) Over eenige nieuwe geslachten uit de familie der Piperaceën. Verslagen en Mededeelingen der Koninlijke Akademie van Wetenschappen. *Afdeeling Natuurkunde* ***1842***, 80-84.

Miquel, F.A.W. (1843b) Piperaceae. *Beiträge zur Botanik* (ed. by Meyen, F.J.F.) *Verhandlungen der Kaiserlichen Leopoldinisch-Carolinischen Akademie der Naturforscher*, **16**, suppl. 2, 483**-**491.

Miquel, F.A.W. (1843c-1844) *Systema Piperacearum*. H. A. Kramers, Rotterdam.

Miquel, F.A.W. (1852) Piperaceae. *Flora Brasiliensis 4* (ed. by Martius, C.F.P.), pp. 7-23. Musei C.R. Pal. Vindobonensis, München & Leipzig.

Miller, K.G., Fairbanks, R.G., Mountain, G.S., (1987) Tertiary oxygen isotope synthesis, sea level history and continental margin erosion. *Paleoceanography*, **2**, 1-19.

Moody, M.L., Hufford, L., Soltis, D.E. & Soltis, P.S. (2001) Phylogenetic relationships of Loasaceae subfamily Gronovioideae inferred from *matK* and ITS sequence data. *American Journal of Botany*, **88**, 326-336.

Moore, M.J., Tye, A. & Jansen, R.K. (2006) Patterns of long-distance dispersal in *Tiquilia* subg. *Tiquilia* (Boraginaceae): Implications for the origin of amphitropical disjuncts and Galápagos islands endemics. *American Journal of Botany*, **93**, 1163-1177.

Moore, M.J., Bell, C.D., Soltis, P.S. & Soltis, D.E. (2007) Using plastid genome-scale data to resolve enigmatic relationships among basal angiosperms. *Proceedings of the National Academy of Sciences,* **104**, 19363-19368.

Moritz, C., Patton, J. L., Schneider, C. J. & Smith, T. B. (2000) Diversification of rainforest faunas: an integrated molecular approach. *Annual Review of Ecology and Systematics,* **31**, 533-563.

Müller, K.F. (2004) PRAP - computation of Bremer support for large data sets. *Molecular Phylogenetics and Evolution*, **31**, 780-782.

Müller, K. (2005) SeqState: primer design and sequence statistics for phylogenetic DNA data sets. *Applied Bioinformatics*, **4**, 65-69.

Müller, K., Quandt, D., Müller, J. & Neinhuis, C. (2005) Phyde®—phylogenetic data editor. Available from: http:\\www.phyde.de.

Nesbitt, T.C. & Tanksley, S.D. (2002) Comparative sequencing in the genus Lycopersicon. Implications for the evolution of fruit size in the domestication of cultivated tomatoes. *Genetics*, **162**, 365-379.

Nichols, R. (2001) Gene trees and species trees are not the same. *Trends in Ecology and Evolution*, **16**, 358-64.

Nickrent, D.L., Blarer, A., Qiu, Y-L., Soltis, D.E., Soltis, P.S. & Zanis, M. (2002) Molecular data place Hydnoraceae with Aristolochiaceae. *American Journal of Botany*, **89**, 1809-1817.

Noble, D.C., McKee, E.H., Mourier, T. & Mégard, F. (1990) Cenozoic stratigraphy, magmatic activity, compressive deformation and uplift in northern Peru. *Geological Society of America Bulletin*, **102**, 1105-1113.

Nyffeler, R. & Rowley, G.D. (2002) Genus *Peperomia*. *Illustrated handbook of succulent plants* (ed. by Eggli, U.), Springer Verlag, Heidelberg.

Page, R.D.M. (1998) GeneTree: comparing gene and species phylogenies using reconciled trees. *Bioinformatics*, 14, 819-820.

Parks, M., Cronn, R., Liston, A. (2009) Increasing phylogenetic resolution at low taxonomic levels using massively parallel sequencing of chloroplast genomes. *BMC Biology*, **7**, 84.

Pennington, R.T. & Dick, C.W. (2004) The role of immigrants in the assembly of the South American rainforest tree flora. *Philosophical Transactions of the Royal Society of London Series B, Biological Sciences*, **359**, 1611-1622.

Pennington, R.T., Prado, D.A. & Pendry, C. (2000) Neotropical seasonally dry forests and Pleistocene vegetation changes. *Journal of Biogeography.* **27**, 261-273.

Pennington, R.T., Lavin, M., Prado, D.E., Pendry, C.A., Pell, S.K. & Butterworth, C.A. (2004) Historical climate change and speciation: neotropical seasonally dry forest plants show patterns of both Tertiary and Quaternary diversification. *Philosophical Transactions of the Royal Society of London Series B, Biological Sciences*, **359**, 515-538.

Pino, G. (2004) *Peperomias de Cajamarca*. published by the author, Lima.

Pino, G. (2008) Three new succulent Peperomias from Peru. *Cactus and Succulent Journal*, **80**, 232-239.

Pino, G. (2010) Alte und neue Namen der sukkulenten Arznei-Peperomias aus Peru. *Avonia*, **28**, 39-55.

Pino, G., Klopfenstein, O. & Cieza N. (2005) Four new Peperomias from northern Peru. *Haseltonia*, **11**, 103-115.

Posada, D. (2008) jModelTest: Phylogenetic Model Averaging. *Molecular Biology and Evolution*, **25**, 1253-1256.

Rahbek, C. & Graves, G.R. (2001). Multiscale assessment of pat- terns of avian species richness. *Proceedings of the National Academy of Sciences, USA*, **98**, 4534–4539.

Rambaut, A. (2009) FigTree v1.2.3. Available from: http://tree.bio.ed.ac.uk/software/ figtree/.

Rambaut, A. & Drummond, A.J. (2009). Tracer v1.5. Available from: http:// beast.bio.ed.ac.uk/Tracer.

Rauh W. (1989) *Peperomia macrorhiza* H.B.K. A little known tuberous species from northern Peru. *Cactus and Succulent Journal*, **61**, 225-232.

Rauh, W. & Kimnach, M. (1987) *Peperomia dolabella*, eine bemerkenswerte neue, knollenbildende *Peperomia* aus Nordperu. *Kakteen und andere Sukkulenten*, **38**, 63-66.

Raven, P.H. (1963) Amphitropical Relationships in the Floras of North and South America. *The Quarterly Review of Biology*, **38**, 151-177.

Raven, P.H. & Axelrod D.I. (1974) Angiosperm Biogeography and past continental movements. *Annals of the Missouri Botanical Garden,* **61**, 539-673.

Ree, R.H. & Smith, S.A. (2008) Maximum likelihood inference of geographic range evolution by dispersal, local extinction, and cladogenesis. *Systematic Biology*, **57**, 4-14.

Ree, R.H., Moore, B.R., Webb, C.O. & Donoghue, M.J. (2005) A likelihood framework for inferring the evolution of geographic range on phylogenetic trees. *Evolution*, **59**, 2299-2311.

Renner, S.S. (2005) Relaxed molecular clocks for dating historical plant dispersal events. *Trends in Plant Science*, **10**, 550-558.

Richardson, J.E., Chatrou, L.W., Mols, J.B., Erkens, R.H.J. & Pirie, M.D. (2004) Historical biogeography of two cosmopolitan families of flowering plants: Annonaceae and Rhamnaceae. *Philosophical Transactions of the Royal Society of London Series B, Biological Sciences*, **359**, 1495-1508.

Richter, M. Diertl, K.-H., Emck, P., Peters, T. & Beck, E. (2009) Reasons for an outstanding plant diversity in the tropical Andes of Southern Ecuador. *Landscape Online*, **12**, 1–35.

Ronquist, F. (1996) DIVA, version 1.1. Computer program and manual available by anonymous FTP from Uppsala University, Uppsala, Sweden. Available at: ftp.uu.se or ftp.systbot.uu.se.

Ronquist, F. (1997) Dispersal-vicariance analysis: a new approach to the quantification of historical biogeography. *Systamatic Biology*, **45**, 195-203.

Ruiz H. & Pavón J. (1794) *Florae Peruvianae et Chilensis Prodromus*. Gabrielis de Sancha, Madrid.

Ruiz H. & Pavón J. (1798a) *Systema Vegetabilium Florae Peruvianae et Chilensis part 1*,pp. 10-22. Gabrielis de Sancha, Madrid.

Ruiz H. & Pavón J. (1798b) *Flora peruviana et chilensis*, pp. 29-33. Gabrielis de Sancha, Madrid.

Rzedowski, J. (2006). Vegetación de México.*1ra Edición digital, Comisión Nacional para el Conocimiento y Uso de la Biodiversidad*, pp 504, México.

Samain, M.S., Mathieu, G., Vanderschaeve, L., Wanke, S., Neinhuis, C. & Goetghebeur, P. (2007) Nomenclature and typification of the subdivisional names of the genus *Peperomia* (Piperaceae). *Taxon*, **56**, 229-236.

Samain, M.S. (2008) *Tackling Pandora's Box. Order out of chaos in the giant genus Peperomia (Piperaceae)*. Ghent University, Belgium.

Samain, M.S., Vanderschaeve, L., Chaerle, P., Goetghebeur, P., Neinhuis, C. & Wanke, S. (2009) Is morphology telling the truth about the evolution of the species rich genus Peperomia (Piperaceae)? *Plant Systemetics and Evolution*, **278**, 1-21.

Samain, M.S., Mathieu, G., Pino, G., Symmank, L., Cieza, N., Neinhuis, C., Goetghebeur P. & Wanke S. (in press) The geophytic *Peperomia* subgenus *Tildenia* (Piperaceae) in the Andes with the description of new species in a phylogenetic framework. *Plant Ecology and Evolution*, **5**, in press.

Sanderson, M.J. (2002) Estimating absolute rates of molecular evolution and divergence times: a penalized likelihood approach. *Molecular Biology and Evolution*, **19**, 101-109.

Sanderson, M.J., Thorne, J.L., Wikstrom, N. & Bremer, K. (2004) Molecular evidence on plant divergence times. *American Journal of Botany*, **91**,1656-1665.

Sang, T., Crawford, D.J. & Stuessy,T.F. (1997) Chloroplast DNA phytogeny, reticulate evolution, and biogeography of Paeonia (Paeoniaceae). *American Journal of Botany*, **84**, 1120-1136.

Sang, T & Zhang, D. (1999) Reconstructing hybrid speciation using sequences of Low Copy Nuclear Genes: hybrid origins of five Paeonia species based on *Adh* gene phylogenies. *Systematic Botany*, **24**, 148-163.

Sang, T. (2002) Utility of low-copy nuclear gene sequences in plant phylogenetics. *Critical Reviews in Biochemistry and Molecular Biology*, **37**,121-147.

Sanmartín, I. & Ronquist, F. (2004) Southern Hemisphere Biogeography Inferred by Event-Based Models: Plant versus Animal Patterns. *Systematic Biology*, **53**, 216-243.

Santos, C., Jaramillo, C., Bayona, G., Rueda, M., & Torres, V. (2008) Late Eocene marine incursion in northwestern South America. *Palaeogeography, Palaeoclimatology, Palaeoecology*, **264**, 140-146.

Shaw, J., Lickey, E.B., Beck, J.T., Farmer, S.B., Liu, W., Miller, J., Siripun, K.C., Winder, C.T., Schilling, E.E. & Small, R.L: (2005) The tortoise and the hare II: relative utility of 21 noncoding chloroplast DNA sequences for phylogenetic analysis. *American Journal of Botany*, **92**, 142-166.

Shaw, J., Lickey, E.B., Schilling, E.E. & Small, R.L. (2007) Comparison of whole chloroplast genome sequences to choose noncoding regions for phylogenetic studies in angiosperms: the tortoise and the hare III. *American Journal of Botany*, **94**, 275-288.

Shimodaira, H. & Hasegawa, M. (1999) Multiple comparisons of log-likelihoods with applications to phylogenetic inference. *Molecular Biology and Evolution*, **16**, 114-1116.

Schultheis, L.M., Baldwin, B.G (1999) Molecular phylogenetics of Fouquieriaceae: evidence from nuclear rDNA ITS studies. *American Journal of Botany*, **86**, 578-589.

Simmons, M.P. & Ochoterena, H. (2000) Gaps as characters in sequence-based phylogenetic analyses. *Systematic Biology,* **49**, 369-381.

Simpson, G.G. (1980) *Splendid isolation. The curious history of South American mammals.* Yale University Press, New Haven.

Simpson, B.B., Tate, J.A. & Weeks, A. (2005) The biogeography of *Hoffmannseggia* (Leguminosae, Caesalpinioideae, Caesalpinieae): a tale of many travels. *Journal of Biogeography*, **32**, 15-27.

Small, R.L., Ryburn, J.A., Cronn, R.C., Seelanan, T. & Wendel, J.F: (1998) The tortoise and the hare: choosing between non-coding plastome and nuclear Adh sequences for phylogeny reconstruction in a recently diverged plant group. *American Journal of Botany*, **85**, 301-1315.

Smith, J.F., Stevens, A.C., Tepe, E.J. & Davidson, C. (2008) Placing the origin of two species-rich genera in the late Cretaceous with later species divergence in the Tertiary: a phylogenetic, biogeographic and molecular dating analysis of *Piper* and *Peperomia* (Piperaceae). *Plant Systematics and Evolution*, **275**, 9-30.

Smith, S.A., Beaulieu, J.M. & Donoghue, M.J. (2010) An uncorrelated relaxed- clock analysis suggests an earlier origin for flowering plants. *Proceedings of the National Academy of Sciences,* **107**, 5897-5902.

Smith, S.Y. & Stockey, R.A. (2007) Establishing a fossil record for the perianthless Piperales: *Saururus tuckerae* sp. nov. (Saururaceae) from the Middle Eocene Princeton Chert. *American Journal of Botany*, **94**, 1642-1657.

Soltis, P. & Soltis,D. (2000) The role of genetic and genomic attributes in the success of polyploids. *Proceedings of the National Academy of Sciences*, **97**, 7051-7057.

Somoza, R. (1998) Updated Nazca (Farallon)-South America relative motions during the last 40 Myr: implications for mountain building in the central Andean region. *Journal of South American Earth Science*, **11**, 211-215.

Steele, K.P. & Vilgalys, R. (1994) Phylogenetic analyses of Polemoniaceae using nucleotide sequences of the plastid gene *mat*K . *Systematic Botany*, **9**, 126–142.

Stehli, F. & Webb, S. (1985) The Great American Biotic Interchange. *Plenum Press*, New York.

Stern, S.R., Tepe, E.J. & Bohs, L.A. (2008) Checklist of *Solanum* of north-central Peru: a hotspot of biological diversity. *Arnoldoa,* **15**, 277-284.

Stöver, B.C. & Müller, K.F. (2010) TreeGraph 2: Combining and visualizing evidence from different phylogenetic analyses. *BMC Bioinformatics*, **11**, 7.

Strand, A.E., Leebens-Mack, J. & Milligan, B.G. (1997) Nuclear DNA-based markers for plant evolutionary Biology. *Molecular Ecology*, **6**,113-118.

Struwe, L., Haag, S., Heiberg, E. & Grant, J. R. (2009) Andean speciation and vicariance in neotropical *Macrocarpaea* (*Gentianaceae-Helieae*). *Annals of the Missouri Botanical Garden,* **96**, 450-469.

Swofford, D.L. (2003) PAUP* Phylogenetic Analysis using Parsimony (*and other Methods), Version 4. Sunderland, MA: Sinauer Associates.

Sytsma, K.J. & Walker J. (2005) The 'giant genus' problem: issues, solutions, opportunities. *XVII Internacional Botanical Congress*, Vienna, Austria, 17-23 July 2005, Abstract.

Tank, D. & Sang, T. (2001) Phylogenetic utility of the glycerol-3–phosphate acyltransferase gene: Evolution and implications in Paeonia (Paeoniaceae). *Molecular Phylogenetics and Evolution*, **19**, 421-429.

Thomas, M.M., Garwood, N.C., Baker, W.J., Henderson, S.A., Russell, S.J., Hodel, D.R. & Bateman, R.M. (2006) Molecular phylogeny of the palm genus Chamaedorea, based on the low-copy nuclear genes PRK and RPB2. *Molecular Phylogenetics and Evolution*, **38**, 398-415.

Toledo, V.M. (1976) *Los cambios climáticos del Pleistoceno y sus efectos sobre la vegetación tropical cálida y húmeda de México.* Master thesis, Universidad Nacional Autónoma de México, Mexico City.

Torsvik, T.H. & van der Voo, R. (2002) Refining Gondwana and Pangea palaeogeography: estimates of Phanerozoic non-dipole (octupole) fields. *Geophysical Journal International*, **151**, 771-794.

Trelease, W. (1922) The peltate *Peperomias* of North America. *Botanical Gazette*, **73**, 133–147.

Trelease, W. (1930) The geography of American peppers. *Proceedings of the American Philosophical Society*, **69**, 309-327.

Trelease, W. (1936) Piperaceae. *Flora of Peru,* Botanical Series 13 (ed. by Macbride, J.F.). pp. 3-253. Field Museum of Natural History, Chicago.

van der Hammen, T. (1974) The Pleistocene changes of vegetation and climate in tropical South America. *Journal of Biogeography*, **1**, 3-26.

van der Hammen, T. & Hooghiemstra, H. (2000) Neogene and Quaternary history of vegetation. *Quaternary Science Reviews*, **19**, 725-742.

Vargas, P., Baldwin, B.G. & Constance, L. (1998) Nuclear ribosomal DNA evidence for a western North American origin of Hawaiian and South American species of Sanicula (Apiaceae). *Proceedings of the National Academy of Sciences, USA*, **95,** 235-240.

Véliz, M. (2007) Nuevas especies del género *Peperomia* (Piperaceae) de Centro América. *Cactus Aventuras Internacional*, **73**, 2–13.

von Hagen, K.B. & Kadereit, J.W. (2001) The phylogeny of *Gentianella* (Gentianaceae) and its colonization of the southern hemisphere as revealed by nuclear and chloroplast DNA sequence variation. *Organisms Diversity & Evolution*, **1**, 61-79.

von Hagen, K.B. & Kadereit , J.W. (2003) The diversification of *Halenia* (Gentianaceae): ecological opportunity versus key innovation. *Evolution*, **57**, 2507-2518.

Vovides, A.P., Pérez-Farrera, M.A., González-Astorga J. & Iglesias C. (2008) A new species of *Ceratozamia* (Zamiaceae) from Oaxaca, Mexico with comments on habitat and relationships. *Botanical Journal of the Linnean Society*, **157**, 1691-75.

Wahlberg, N., West & Wheat, C. (2008) Genomic Outposts Serve the Phylogenomic Pioneers: Designing Novel Nuclear Markers for Genomic DNA Extractions of Lepidoptera. *Systematic Biology*, **57**, 231-242.

Wall, P.K., Leebens-Mack, J., Müller, K.F., Dawn, F., Altman. N.S. & dePamphilis CW (2008) PlantTribes: a gene and gene family resource for comparative genomics in plants. *Nucleic Acids Research*, **36**, D970–D976.

Wang, H.C., Moore, M.J., Soltis, P.S., Bell, C.D., Brockington, S.F., Alexandre, R., Davis, C.C., Latvis, M., Manchester, S.R. & Soltis, D.E.(2009) Rosid radiation and the rapid rise of angiosperm-dominated forests. *Proceedings of the National Academy of Sciences*, **10**, 3853-3858.

Wanke, S., Samain, M.S., Vanderschaeve, L., Mathieu, G., Goetghebeur, P. & Neinhuis, C. (2006) Phylogeny of the genus *Peperomia* (Piperaceae) inferred from the *trnK/matK* region (cpDNA). *Plant Biology*, **8**, 93-102.

Wanke, S., Jaramillo, M.A., Borsch, T., Samain, M.S., Quandt, D. & Neinhuis, C. (2007) Evolution of Piperales - *matK* gene and *trnK* intron sequence data reveal lineage specific resolution contrast. *Molecular Phylogenetics and Evolution*, **42**, 477-497.

Weigend, M. (2002) Observations on the biogeography of the Amotape-Huancabamba Zone in Northern Peru. *The Botanical Review*, **68**, 38-54.

Weigend, M. (2004) Additional observations on the biogeography of the Amotape-Huancabamba zone in Northern Peru: Defining the South-Eastern limits. *Revista Peruana de Biología,* **11**, 127-134.

Weigend, M., Cano, A., Rodríguez, R.E. & Breitkopf, H. (2010a) Four new species of *Ribes* (Grossulariaceae), primarily from the Amotape-Huancabamba Zone in Northern Peru. *Novon*, **20**, 228-238.

Weigend, M., Gottschling, M., Hilger, H.H. & Nürk, N.M. (2010b) Five new species of *Lithospermum* L. (Boraginaceae tribe Lithospermeae) in Andean South America: Another radiation in the Amotape-Huancabamba Zone. *Taxon*, **59**, 1161-1179.

Wendt, T. (1987) Las selvas de Uxpanapa, Veracruz-Oaxaca, México: evidencia de refugios florísticos Cenozoicos. *Anales Del Instituto de Biología UNAM* (Series Bot.), **58**, 29-54.

Westerhold, T., Bickert, T. & Rohl, U. (2005) Middle to late Miocene oxygen isotope stratigraphy of ODP site 1085 (SE Atlantic): new constrains on Miocene climate variability and sea-level fluctuations. *Palaeogeography, Palaeoclimatology, Palaeoecology*, **217**, 205-222.

Wicke, S., Schneeweiss, G.M., Mueller, K.F., dePamphilis, C.W. & Quandt, D. (2011) The evolution of the plastid chromosome in land plants: gene content, gene order, gene function. *Plant Molecular Biology*, DOI 10.1007/s11103-011-9762-4.

Wiegmann, B., Trautwein, M,, Kim, J., Cassel, B., Bertone, M., Winterton, S. & Yeates, D. (2009) Single-copy nuclear genes resolve the phylogeny of the holometabolous insects. *BMC Biology*, **7**, 34.

Wilf, P., Cúneo, N.R., Johnson, K.R., Hicks, J.F., Wing, S.L. & Obradovich, J.D. (2003) High plant diversity in Eocene South America: evidence from Patagonia. *Science*, **300**, 122-125.

Whittall, J.B., Medina-Marino, A., Zimmer, E.A. & Hodges, S.A. (2006) Generating single copy nuclear gene data for a recent adaptive radiation *Molecular Phylogenetics and Evolution*, **39**, 124-134.

Wu, F., Mueller, L.A., Crouzillat, D., Petiard, V. & Tanksley, S.D. (2006) Combining bioinformatics and phylogenetics to identify large sets of single-copy orthologous genes (COSII) for comparative, evolutionary and systematic studies: A test case in the Euasterid plant clade. *Genetics,* **174**, 1407-1420.

Young, K.R. & Reynel, C. (1997) Huancabamba Region, Peru and Ecuador. *Centres of plant diversity: a guide and strategy for their conservation, 3: North America, Middle America, South America, Caribbean Islands* (ed. by Davis,S.D., Heywood, V.H., Herrera-MacBryde, O., Villa-Lobos, J. and Hamilton, A.C.). pp. 465-469. IUCN, Cambridge.

Youngsteadt, E., Nojima, S., Häberlein, C., Schulz, S. & Schal C. (2008) Seed odor mediates an obligate ant-plant mutualism in Amazonian rainforests. *Proceedings of the National Academy of Sciences,* **105**, 4571-4575.

Yuncker, T.G. (1965) A new *Peperomia* from Mexico. *Kew Bulletin*, **19**, 415.

Zachos, J., Pagani, M., Sloan, L., Thomas, E. & Billups, K. (2001) Trends, rhythms, and aberrations in global climate 65 Ma to present. *Science*, **292**, 686-693.

Zavada, M.S. & Benson, J.M. (1987) First fossil evidence for the primitive angiosperm family Lactoridaceae. *American Journal of Botany*, **74**,1590-1594.

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1992-1999	high school
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2000-2007	Dresden University of Technology
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2008	Two month field trip to Bolivia in cooperation with “Herbario Nacional de Bolivia” Topic: “Morphology and Phylogeography of the genus Peperomia in Bolivia”
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PUBLICATION LIST

Publications

Samain, M-S., Mathieu, G., Pino, G., **Symmank, L**., Cieza, N., Neinhuis, C., Goetghebeur P. & Wanke S. (2011) The geophytic *Peperomia* subgenus *Tildenia* (Piperaceae) in the Andes with the description of new species in a phylogenetic framework. *Plant Ecology and Evolution*, 144, 148-176.

Mathieu, G., **Symmank, L**., Callejas, R., Wanke, S., Neinhuis, C., Goetghebeur, P. & Samain, M-S. (2011) New geophytic *Peperomia* (Piperaceae) species from Mexico, Belize and Costa Rica. *Revista Mexicana de Biodiversidad*, 82, 357-382.

Symmank, L., Samain, M-S, Smith, J.F., Pino, G., Stoll, A., Goetghebeur, P., Neinhuis, C. & Wanke, S. The extraordinary journey of *Peperomia* subgenus *Tildenia* (Piperaceae): insights into diversification and colonization patterns from its cradle in Peru to the Trans-Mexican Volcanic Belt. *Journal of Biogeography*, in press.

Congress contributions

Shaw, B., **Symmank, L.**, Neinhuis, C., Frahm, J.P., Quandt D. & Shaw, J. (2007) Phylogeny and biogeography of the cosmopolitan moss genus *Philonotis* (Bartramiaceae). *Bryoecology,* Tasmania.

S**ymmank, L.**, Shaw, B., Neinhuis, C., Frahm, J.P. & Quandt D. (2007) Phylogenetic relationships among apple mosses (Bartramiaceae)“.*125 Jahre Deutsche Botanische Gesellschaft*“ *Botanikertagung 2007.* Hamburg/Germany.

Symmank, L., Samain, M.-S., Goetghebeurm P., Mathieum G., Neinhuism C. & Wanke S. (2008) Independent evolution of different terrestrial life forms in the genus *Peperomia* (Piperaceae) and implications for biogeography of the tuberous species. *Systematics 2008*, Göttingen/Germany.

Samain, M. S., **Symmank, L.,** Mathieu, G., Neinhuis, C., Goetghebeur, P. & Wanke S. (2008) Evolution of terrestrial life forms in the genus *Peperomia* (Piperaceae): a result of orogeny of the Andes and the Sierra Madre, or radiation out of ice age refugia? *Xth Symposium of the International Organisation of Plant Biosystematists* Strbske Pleso, Vysoké Tatry Mountains/Slovakia.

Symmank, L., Samain, M.-S., Goetghebeur, P., Mathieu, G., Neinhuis, C. & Wanke S. (2008) Evolution of terrestrial life forms in the genus *Peperomia* (Piperaceae). *Botany 2008*, Vancouver/Canada.

Stoll, A., Hahn, S. & **Symmank, L** (2009) ¿Cuantas orejas tiene el zorro? Advances in taxonomical revision of the genus Aristolochia in Chile. *IV Reunion de Biologia Vegetal*, La Serena/Chile.

Simon, J., **Symmank, L.**, Samain, M-S., Neinhuis, C., dePamphilis, C.W. & Wanke, S. (2010) Chasing the hare – Evaluating the utility of a nuclear single copy gene region for evolutionary biological questions at and below the species level. *Botany 2010*, Providence/USA.

Stoll A., **Symmank L.**, Hahn S. (2010) ¿Cuantas orejas tiene el zorro? Avances en la revisión taxónomica del género *Aristolochia* en Chile. *X Congreso Latinoamericano de Botánica*, La Serena/Chile.

Samain, M.-S., **Symmank, L.**, Pino-Infante, G., Goetghebeur, P., Neinhuis, C. & Wanke S. (2010) Hacia la resolución de un rompecabezas gigante. Evolución, biogeografía y sistemática de *Peperomia* (Piperaceae), uno de los mas grandes géneros de angioespermas. *X Congreso Latinoamericano de Botánica,* La Serena/Chile.

Damayanti, L., Wicke, S., **Symmank, L**., Muñoz, J., Frahm, J.P., Shaw, B. & Quandt D. (2010) Gone with the wind: the phylogeography of Strictidium. *19th International Symposium "Biodiversity and Evolutionary Biology"*, Vienna/Austria.

Erklärung

Hiermit versichere ich, dass ich die vorliegende Arbeit ohne unzulässige Hilfe Dritter und ohne Benutzung anderer als der angegebenen Hilfsmittel angefertigt habe; die aus fremden Quellen direkt oder indirekt übernommenen Gedanken sind als solche kenntlich gemacht. Die Arbeit wurde bisher weder im Innland noch im Ausland in gleicher oder ähnlicher Form einer anderen Prüfungsbehörde vorgelegt. Die Bestimmungen der Promotionsordnung sind mir bekannt.